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(54) Microbiological Process for Producing Agrochemically Usable Active Ingredients

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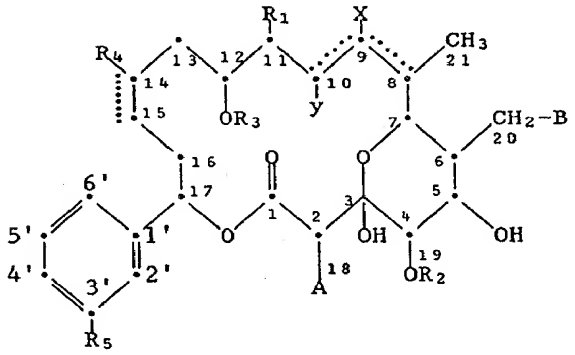
# Microbiological process for producing agrochemically usable active ingredients


## Abstract

A description is given of a microbiological process for producing compounds of the formula I



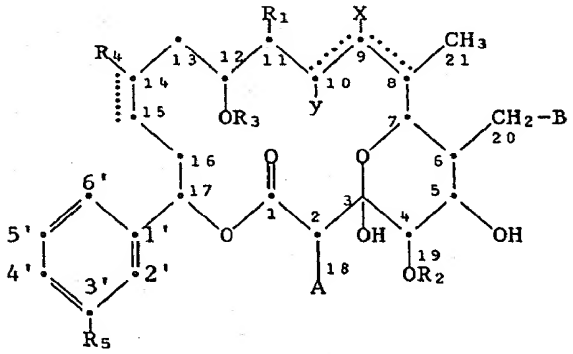
(I)

which can be used in agrochemistry for combating or preventing plant diseases. The substituents have the meaning given in the description. Six new microorganisms are described for carrying out the process.

5-17208/210/+/GBF

# Microbiological process for producing agrochemically usable active ingredients

The present invention relates to a microbiological process for producing agrochemically usable macrocyclic active ingredients of the formula I, novel microorganisms for carrying out the process and also the active ingredients prepared by the process or the substrate prepared by the process which contains such active ingredients, and the use of the active ingredients for combating and preventing plant diseases.



(I)

The above formula relates to the following substituent combinations, the macrocyclic ring either being saturated or, alternatively, containing a double bond in the 8,9-position or in the 9,10-position or in the 14,15-position:

| Comp. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | A | B | X | Y | Double bond |
|---|---|---|---|---|---|---|---|---|---|---|
| A | $OCH_3$ | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | H | H | Δ9,10 |
| B | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | H | H | -- |
| C | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | H | H | Δ9,10 |
| D | OH | $CH_3$ | $CH_3$ | H | H | H | H | H | H | -- |
| E | $OCH_3$ | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | OH | H | -- |
| F | $OCH_3$ | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | H | H | -- |
| H | $OCH_3$ | $CH_3$ | H | H | H | $CH_3$ | H | H | OH | Δ8,9 |
| J | $OCH_3$ | H | $CH_3$ | H | H | $CH_3$ | H | H | H | -- |
| M | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | OH | H | -- |
| N | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | H | $OCH_3$ | Δ9,10 |
| Q | $OCH_3$ | $CH_3$ | $CH_3$ | H | H | H | H | H | H | -- |
| R | OH | $CH_3$ | H | H | H | H | H | H | H | -- |
| S | OH | $CH_3$ | H | H | H | $CH_3$ | H | H | H | Δ9,10 |
| T | OH | $CH_3$ | H | H | H | $CH_3$ | H | H | H | -- |
| U | $OCH_3$ | $CH_3$ | $CH_3$ | OH | H | $CH_3$ | H | H | H | Δ9,10 |
| V | $OCH_3$ | $CH_3$ | H | H | H | $CH_3$ | H | H | H | Δ9,10 |
| X | OH | $CH_3$ | H | H | OH | $CH_3$ | H | H | H | Δ9,10 |
| Y | =O | $CH_3$ | H | H | H | $CH_3$ | H | OH | H | -- |
| Z | $OCH_3$ | $CH_3$ | H | OH | H | $CH_3$ | H | H | H | Δ9,10 |
| β | $OCH_3$ | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | H | H | Δ9,10 |
| γ | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | 9,10-epoxy | | -- |
| δ | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | H | H | Δ9,10 |
| ζ | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | OH | H | H | Δ9,10 |
| η | OH | $CH_3$ | $CH_3$ | OH | H | $CH_3$ | H | H | H | Δ9,10 |
| κ | OH | $CH_3$ | H | H | H | $CH_3$ | H | H | H | Δ9,10 |
| μ | $OCH_3$ | $CH_3$ | H | H | H | $CH_3$ | H | H | OH | -- |
| ν | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | OH | H | Δ14,15 |
| ξ | OH | $CH_3$ | H | H | H | $CH_3$ | H | H | H | Δ9,10 |
| o | $OCH_3$ | $CH_3$ | $CH_3$ | OH | H | $CH_3$ | H | H | H | Δ9,10 |
| π | OH | $CH_3$ | H | H | H | $CH_3$ | H | H | H | Δ9,10 |
| ρ | OH | $CH_3$ | H | H | H | $CH_3$ | H | OH | H | -- |
| σ | $OCH_3$ | $CH_3$ | $CH_3$ | OH | H | $CH_3$ | H | H | H | Δ9,10 |

These 32 compounds are prepared by microbiological cultivation of myxobacteria from the Sorangium/Polyangium group by which they are produced completely or partially and in different ratios as a function of the particular strain. Common to all the strains is the production of the compound A as one of the main products. The compounds of the formula I will be termed here and below "soraphens", and therefore the compounds in the above table are called "soraphen A" to "soraphen σ".

The invention relates, in particular, to 6 novel microorganism strains of the cellulose-decomposing myxobacteria from the Sorangium/Polyangium group which, as is known, can frequently be found in soil samples, veg-

etational material or in animal dung. Characteristic of this group, which is not generally mandatorily classified taxonomically, is their ability to grow on cellulose or cellulose degradation products as the sole source of carbon. Regardless of whether they belong strictly speaking to the Sorangium/Polyangium group or to a taxonomically related sector, the 6 strains according to the invention have, compared with by far the majority of representatives of this group, the characteristic feature that they produce at least one of the "soraphens" of formula I mentioned above, and preferably produce at least two "soraphens", including "soraphen A".

The 6 strains have, here and below, the collective name Sorangium(Polyangium) cellulosum. They originate from soil samples which were collected at different times at different places in Europe, Africa or the USA. They were deposited at the Deutsche Sammlung von Mikroorganismen (DSM) (German Collection of Microorganisms) in Braunschweig, Federal Republic of Germany, in accordance with the Budapest Treaty. They have the following characteristics.

1.) Sorangium(Polyangium) cellulosum, strain "So ce 139", isolated in May 1986 from a soil sample collected in autumn 1982 at Fort Huachaca, Arizona, USA. Depositary number: DSM 5397. Date of deposit: 2nd June 1989.

2.) Sorangium(Polyangium) cellulosum, strain "So ce 170", isolated in April 1987 from a soil sample from the island of Delos, Greece, collected in May 1981. Depositary number: DSM 4795. Date of deposit: 2nd September 1988.

3.) Sorangium(Polyangium) cellulosum, strain "So ce 191", isolated in August 1987 from a soil sample from the island of Madeira, Portugal, collected in February 1987. Depositary number: DSM 4796. Date of deposit: 2nd September 1988.

4.) Sorangium(Polyangium) cellulosum, strain "So ce 192", isolated in August 1987 from a soil sample from Nigeria collected in March 1987. Depositary number: DSM 4797. Date of deposit: 2nd September 1988.

5.) Sorangium(Polyangium) cellulose, strain "So ce 231", isolated in April 1988 from a soil sample collected in April 1987 at Didyma, Turkey, Depositary number: DSM 5393. Date of deposit: 2nd June 1989.

6.) Sorangium(Polyangium) cellulosum, strain "So ce 242", isolated in October 1988 from a soli sample collected in April 1988 at Possuoli, Italy. Depositary number: DSM 5414. Date of deposit: 19th June 1989.

In the following, the name Sorangium cellulosum will be used for these strains.

The process according to the invention for producing a "soraphen" of the formula I comprises aerobically culturing one of the strains Sorangium cellulosum, "So ce 139", "So ce 170", "So ce 191", "So ce 192", "So ce 231" or "So ce 242" or a clone, mutant etc. derivable from them, in a suitable nutrient medium and separating the "soraphen" formed therefrom.

European Patent Application EP-A-282,455 published on September 14, 1988 describes another Sorangium cellulosum microorganism "So ce 26" whose fermentation yields the soraphens A and B mentioned above. The present invention does not relate to these two products and their sole use in agents.

The present invention relates to further valuable microbicidal fermentation products for plants which are produced by the microorganism "So ce 26" (depositary number NCIB 12,411)

A

and/or by the microorganisms "So ce 139", "So ce 170", "So ce 191", "So ce 192", "So ce 231" or "So ce 242" claimed herein.

In drawings which illustrate aspects and embodiments of the present invention figure 1 shows a chart outlining separation steps by which soraphen A to soraphen Q can be isolated. Figure 2 shows a chart outlining separation steps by which further soraphens can be isolated.

General process conditions for culturing the 6 production strains

The Sorangium cellulosum strains may be cultured in suitable nutrient media by standard biological methods, e.g. in shake cultures or in fermenters. The fermentation temperature is, as a rule, 10-40°C, preferably 10-35°C and particularly preferably 30°-32°C, and the pH is 6-8, preferably 7.4. The process proceeds aerobically and under sterile conditions.

The composition of the nutrient medium may be varied within fairly large ranges. For nutrients to be assimilated essentially, a carbon source and a nitrogen source and also a source of inorganic mineral salts which include P, S, Mg, K, Fe, Ca, must be present.

The C sources used in fermentation processes are preferably glucose, starch and cellulose, and also their degradation products (for example cellobiose) and also in addition disaccharides, glycerol, acetic acid and others. Suitable N sources are, for example, $NH_4$, $NO_3$ or also peptones. An organic compound as an N source cannot, as a rule, simultaneously be the sole C source and energy source in the fermentation.

Suitable mineral salts are chlorides, nitrates, sulfates, carbonates and phosphates of the elements Na, K, $NH_4$, Mg, Fe and Ca, and in addition Cu, Mn, Mo, Zn, Co and others may be present as trace elements. As far as possible, those salts present may also be bound to ethylenediaminetetraacetic acid (EDTA).

The microorganism culture is introduced into the shake culture or into the fermenter in an initial inoculation amount of 0.1-20 %, preferably 0.5-10 %, very particularly preferably 0.5-5 % (v/v). The duration of culturing is about 2-7 days at approx. 30°C, large-volume batches more rarely up to 10 days or longer. For large-volume batches, smaller precultures are expediently initially fermented. The use of the Sorangium cellulosum strains is possible also in immobilized form, for example in the form of carrier-immobilized cells on alginate.

A derivable clone in the above meaning is any culture which has the features of the deposited clone essential for carrying out the process according to the invention, in particular a culture whose microorganisms contain the same structural genes as the structural genes, responsible for the formation of the compound of the formula I, of the strains "So ce 139", "So ce 170", "So ce 191", "So ce 192", "So ce 231" and "So ce 242". A derivable clone is also any culture whose microorganisms contain a possible equivalent, due to degeneration of the genetic code, of the structural genes, responsible for the formation of compound I, of the 4 strains. A derivable clone is, in particular, any culture which contains cellulose-decomposing myxobacteria, preferably of the species Sorangium cellulosum which are capable of producing the compound of the formula I. The term "derivable clone" of the strains DSM 5397, DSM 4795, DSM 4796, DSM 4797, DSM 5393 and DSM 5414 also includes all the mutants or recombinants which are capable of producing compound I.

The culturing takes place aerobically, that is to say, for example, in a stationary surface culture or, preferably, immersed with shaking or stirring with air or oxygen in shake cultures or in fermenters. Preferably, culturing is carried out in stages, i.e. one or more precultures are first produced in liquid nutrient media which are then transferred by inoculation, for example in a ratio of 1 : 20, to the production medium itself.

The "soraphen" is separated by physico-chemical means using separation methods known per se such as filtration, in particular, however, solvent extraction, and chromatography, in particular adsorption and partition chromatography, and, possibly, crystallization.

The compounds of the formula I can be extracted from the aqueous fermentation broth with lipophilic organic solvents, for example with ketones such as methyl ethyl ketone, cyclohexanone; with alcohols of medium chain length such as isobutanol, pentanol, hexanol; with $C_1$-$C_6$-alkyl acetates (ethyl acetate, propyl acetate, butyl acetate, isobutyl acetate etc.); with toluene, dichloromethane, 1,2-dichloroethane, chlorobenzene, dichlorobenzene etc.

The compounds can be readily extracted from the filtered cell cake with alcohols or ketones (for example methanol, ethanol, acetone, methyl ethyl ketone).

Compounds of the formula I which can be separated by fractional crystallization or other chromatographic separation processes into the soraphens A to ρ (= rho), can then be prepared from the particular extract by concentration and/or precipitation.

Advantageously, the fermentation operation for preparing the compounds of the formula I concludes with the addition of an adsorbent resin which adsorbs the desired products, or the fermentation is carried out from the start in the presence of such an adsorbent resin. Possible adsorbent resins are, in particular, neutral organic polymeric substances, particularly nonionic hydrophobic adsorbent resins which are suitable for lipophilic extraction. The compound of the formula I is bound almost quantitatively to the latter. Examples of such resins are semipolar acrylic ester resins, nonpolar polystyrene/divinylbenzene resins and, particularly, crosslinked polystyrene. Such resins are added in amounts of 0.1 to 5 % (v/v), preferably 0.5 to 2 % (v/v), of the fermentation volume. Active charcoal is also suitable. Particularly advantageous industrially are polystyrene resins such as, for example, XAD-1180* or XAD-16* (manufacturer: Rohm and Haas) which are in filterable form (grains or pellets). After completion of the fermentation the resin is filtered off, washed with water and treated with methanol or ethanol. The alcoholic extract is concentrated. The compound IA (= soraphen A), which largely crystallizes out, can be separated off by adding diethyl ether, ethyl acetate or butyl acetate. The filtrate is purified chromatographically to obtain residual soraphen A, in particular, however, to obtain the residual soraphens B to σ (sigma).

IA

The invention relates to the compounds of the formula I in pure form or in crystallized form. The invention relates, however, also to biomasses, crude extracts or adsorbent resins from the fermentation which contain

* Trade Mark

the compound of the formula I and can be used as such or in further formulated form to combat plant diseases. Biomasses may also find subsequent use or be marketed as ground or pressed dry substances ("cake").

The production processes described, including all the substeps, are a component of the present invention.

In particular, the invention relates to a process for producing soraphen of the formula I which comprises aerobically culturing one of the strains of Sorangium cellulosum "So ce 139", "So ce 170", "So ce 191", "So ce 192", "So ce 231" and "So ce 242" or a microorganism which contains the same structural genes as the structural genes responsible for the formation of soraphen, of the said strains in an aqueous nutrient medium containing a carbon source and nitrogen source and also inorganic salts and isolating the soraphens of the formula I.

The invention primarily relates to an embodiment of the process mentioned above wherein a soraphen-forming microorganism from the group of the cellulose-decomposing myxobacteria is cultured.

A preferred embodiment of the abovementioned process comprises culturing one of the strains Sorangium cellulosum "So ce 139", "So ce 170", "So ce 191", "So ce 192", "So ce 231", "So ce 242" or a soraphen-forming mutant of this strain.

A particularly preferred embodiment of the abovementioned process comprises culturing one of these strains, deposited in accordance with the Budapest Treaty, "So ce 139", "So ce 170", "So ce 191", "So ce 192", "So ce 231", or "So ce 242".

Preferably, the fermentation is carried out under the conditions described in the example section.

Microorganisms which contain the same structural genes responsible for the formation of soraphen as the 6 strains can be produced synthetically, for example by gene manipulation, by isolating the corresponding struc-

tural genes from the 6 strains and incorporating them at a suitable point in the gene material of another suitable microorganism. Suitable microorganisms are those in which the structural genes involved cannot only be incorporated but in which these structural genes are also expressed and in which the soraphen formed is not degraded again, but is preferably excreted into the fermentation broth. Such suitable microorganisms are primarily other strains of the myxobacteria, in particular those of the Sorangium cellulosum species, provided they do not already have the abovementioned structural genes.

Soraphen-forming mutants may, for example, be produced by exposure to ultraviolet rays or X-rays or to chemical mutagens, for example N-methyl-N'-nitro-N-nitrosoguanidine and be isolated by selection according to their specific properties in a manner known per se. Further process measures for producing mutants and recombinants of a microorganism are known to, and familiar to, the person skilled in the art.

The invention relates also to a process for producing a fermentation material which contains soraphen in detectable amount and which comprises culturing one of the strains of Sorangium cellulosum "So ce 139", "So ce 170", "So ce 191", "So ce 192", "So ce 231" and "So ce 242" or a clone derivable from these strains in a nutrient medium and producing soraphen in suitable form. Such culture broths can be used as such or in concentrated form, possibly with the addition of further carrier substances and/or dispersants as agents for combating pathogenic plant microorganisms and are therefore an important part of the present invention.

In a narrower sense, the process comprises culturing one of the microorganisms Sorangium cellulosum "So ce 139", "So ce 170", "So ce 191", "So ce 192", "So ce 231" or "So ce 242" in a culture medium containing at least one assimilatable C source and N source in each case and suitable inorganic salts, at 10-40°C, preferably at 10-35°C, in the presence or absence of an adsorbent resin, then extracting the culture broth or the adsorbent resin filtered off with a suitable solvent phase, concentrating the solution obtained and purifying the residue left, as far as desired, by chromatography and/or recrystallization.

Strain cultures and morphological description:

Strain cultures are kept as plate cultures on VY/2 agar (0.5 % baker's yeast by fresh weight; 0.1 % $CaCl_2$; 1.5 % agar; pH 7.2) or on filter paper (= cellulose source) over ST 21 agar (0.1 % $KNO_3$; 0.1 % $MgSO_4.7H_2O$; 0.1 % $CaCl_2$; 0.1 % $K_2HPO_4$; 0.01 % $MnSO_4.7H_2O$; 0.02 % $FeCl_3$; 0.002 % yeast extract; standard trace element solution[1]; 1 % agar). The plates are incubated at 30°C.

On both media the organisms form swarm colonies which slowly spread across the substrate. Clear differences are observed in detail between the 6 strains.

1) "So ce 139" (DSM 5397): The swarm colony remains largely confined to the filter paper on filter paper over ST 21 agar. In fairly old cultures, the agar cracks at the edge of the filter, and swarm and agar roll themselves in. The filter paper is then completely degraded and in its place a dense field of blackish brown fruiting bodies is found. These are composed of small sporangiola of 15-30 µm in diameter which are in turn coalesced to form small aggregations ranging up to extended unorganized masses. The vegetative cells are slim, cylindrical rods with bluntly rounded ends and measure 0.7-0.9 x 2.5 - 4 µm. In the phase contrast microscope they appear dark, often with bright polar granules. On VY/2 agar they form very large swarm colonies with colourless to orange radial veins and many blackish brown fruiting bodies of similar structure to that described above. There is hardly any attack on the yeast cells of the nutrient medium. The vegetative cells appear somewhat coarser and are about 1 µm thicker.

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[1] 0.02-0.5 mg of Mn salt, Mo salt, Cu salt, Co salt and/or Zn salt per litre [R.Y. Stanier et al. "General Microbiology" 4th ed., p. 36 (1976)]; or 500 mg of EDTA, 300 mg of $FeSO_4.7H_2O$, 3 mg of $MnCl_2.4H_2O$, 5 mg of $CoCl_2.6H_2O$, 1 mg of $CuCl_2.2H_2O$, 2 mg of $NiCl_2.6H_2O$, 3 mg of $Na_2MoO_4.2H_2O$, 5 mg of $ZnSO_4.7H_2O$ and 2 mg of $H_3BO_3$ per litre of distilled water (approx. pH 4) [G. Drews, "Mikrobiolog. Praktikum", 4th ed., p 11, Springer Verlag, Berlin, Heidelberg, New York, Tokyo (1983)].

2) "So ce 170" (DSM 4795): On filter paper over ST 21 agar, the swarm spreads far beyond the filter onto the agar surface. The agar later cracks deeply and the swarm colony rolls itself in. Fruiting bodies may develop on the filter paper: these are composed of small, thick-walled dark-brown sporangiola, generally 20-30 µm in diameter, which are tightly packed together in elongated aggregations generally having sharp boundaries and a diameter of about 50-200 µm. In places continuous extended aggregates of fruiting bodies may appear. On VY/2 agar thick slimy, intensely orange swarm colonies of just under medium size and generally 1-2 cm in diameter are formed. The yeast cells are slowly degraded. Here again the agar cracks deeply in places so that the swarm may drop completely out of the plate. Orange, light brown to dark brown fruiting bodies of similar structure to that above often form on the agar surface. The vegetative cells are coarse rods which are dark in the phase contrast microscope with broadly rounded ends and usually measuring 0.8-1.0 x 2-5 µm. The strain also degrades chitin very efficiently.

3) "So ce 191" (DSM 4796) grows on filter paper over ST 21 agar without spreading onto the agar surface. In this case too, the agar may crack deeply around the edge of the filter paper and the swarm colony may roll itself in. On VY/2 agar large swarms of 6-8 cm in diameter develop which have fine to thick radial veins. There is hardly any attack on the yeast cells. The cylindrical vegetative rods have wide rounded ends, are dark in the phase contrast microscope, are often fairly long and generally measure 0.7-0.9 x 3-8 µm. The strain does not form any fruiting bodies in a pure culture under the culture conditions mentioned. The strain efficiently degrades chitin.

4) "So ce 192" (DSM 4797) spreads extensively over the agar surface when cultured on filter paper over ST 21 agar. The agar may crack deeply and the swarm colony may roll itself in. On the agar surface, particularly, however, on the filter paper, many brightly orange-coloured fruiting bodies develop which consist of tiny sporangiola of usually 10-20 µm in diameter in sharply bounded aggregations having an extension usually of 50-500 µm. Medium-size thick swarms of 2-3 cm in diameter with radial veins and many intensely brownish orange-coloured fruiting bodies having

a similar structure to that described above develop in and on the agar. The vegetative cells are cylindrical, dark rods with wide ends and measure usually 0.7-0.9 x 3-5 µm. The yeast cells are not degraded, but the strain degrades chitin very efficiently.

5) "So ce 231" (DSM 5393) does not spread onto the agar plate in cultures on filter paper over ST 21 agar. The agar may crack at the filter edge but the colonies do not roll themselves in. The filter paper is completely destroyed and in its place a very dense field of intensely brownish orange fruiting bodies is later found. These are of similar structure as in the case of the other strains, but the sporangiola are often arranged in bands. On VY/2 agar very large swarms develop which have radial bundles of fine veins. Brightly orange brown fruiting bodies develop locally. The slim vegetative rods are very delicate and measure 0.6-0.8 x 2.5-6 µm. The yeast cells of the nutrient medium are slowly lysed. The strain also degrades chitin very efficiently.

6) "So ce 242" (DSM 5414) advances on filter paper over ST 21 and also forms fruiting bodies there. The agar cracks deeply at the edge of the filter paper. The filter paper is completely degraded and is replaced by a dense mass of blackish brown fruiting bodies. The sporangiola are often arranged in chains in the latter. Very large swarms with fine radial veins develop on VY/2 agar. The agar cracks in the swarm region at many points in a clawlike manner. In addition, very large numbers of fruiting bodies appear. The yeast cells in the substrate are slowly degraded. The vegetative cells measure 0.7-1.0 µm x 2-4 µm. The strain is very efficient at decomposing chitin.

The strains produce substances which inhibit the growth of numerous yeasts and hyphal fungi. Chemically the inhibitory substances are not only soraphens whose structures have already been elucidated as indicated below. These mixtures of macrocyclic compounds of the soraphen type appear in liquid cultures in the culture supernatant, but may also be isolated from the cells. The antibiotics are produced during the logarithmic phase and the production continues into the steady state growth phase.

All 6 strains have to be adapted to growth in liquid media. They first grow in the form of small, solid, orange-coloured nodules and only gradually form more or less homogeneous cell suspensions after many transfer operations from liquid medium to liquid medium.

Biological characterization of the strains "So ce 139", "So ce 170", "So ce 191", "So ce 192", "So ce 231" and 2So ce 242"

Cellulose degradation: positive
Glucose degradation : positive
Starch degradation : positive
$NH_4$ as N source : positive
$NO_3$ as N source : positive

Table 1: Examples of organisms whose growth is inhibited by culture supernatants of the strains So ce 139, So ce 170, So ce 191, So ce 192, So ce 231 and So ce 242.

| Yeasts: | Rating* |
|---|---|
| Debaryomyces hansenii | strong inhibition |
| Nematospora coryli | strong inhibition |
| Candida albicans | strong inhibition |
| Saccharomyces cerevisiae | medium strong inhibition |
| Rhodotorula glutinis | medium strong inhibition |
| Hansenula anomala | medium strong inhibition |
| Nadsonia fulvescens | no inhibition |
| Torulopsis glabrata | no inhibition |
| Schizosaccharomyces pombe | no inhibition |
| **Hyphal fungi** | |
| Alternaria solani | strong inhibition |
| Pythium debaryanum | strong inhibition |
| Mucor hiemalis | strong inhibition |
| Rhizopus arrhizus | weak inhibition |

* The inhibition values relate to an average culture batch and should be regarded as relative values with respect to one another.

The invention relates to the soraphens C to σ of the formula I in pure form. The invention relates also, however, to biomasses, crude extracts or adsorbent resins from the fermentation which contains the soraphens C to σ of the formula I and are capable of being used as such or in further formulated form for combating plant diseases. Biomasses may also be used further or marketed as ground or pressed dry substances ("cake").

The production process described, including all the substeps are a component of the present invention.

Surprisingly, it has now been found that compounds of the formula I have a very favourable biocidal spectrum against phytopathogenic micro-organisms, in particular against fungi. They have highly advantageous curative, systemic and in particular preventive properties and can be employed for the protection of numerous crop plants. Using the active ingredients of the formula I, pests which occur on plants or parts of plants (fruits, flowers, foliage, stalks, tubers, roots) of various crops can be brought under control or destroyed, additional growth of parts of plants which occurs later also being kept free from phytopathogenic microorganisms.

As microbicides, the active ingredients of the formula I are active, for example, against the phytopathogenic fungi belonging to the following classes: Fungi imperfecti (for example, in particular, Botrytis, furthermore Pyricularia, Helminthosporium, Fusarium, Septoria, Cercospora and Alternaria); Basidiomycetes (for example Rhizoctonia, Hemileia, Puccinia). Moreover, they are active against the class of the Ascomycetes (for example in particular Venturia and Erysiphe, furthermore Podosphaera, Monilinia, Uncinula) and of the Oomycetes (for example Phytophthora, Plasmopara). The compounds of the formula I can furthermore be employed as seed-dressing agents for treating seeds (fruits, tubers, grains) and cuttings in order to protect them from fungal infections, as well as soil-borne phytopathogenic fungi.

The invention also relates to the agents which contain one or more of the soraphens C to σ of the formula I as the active ingredient, in particular plant-protecting agents, as well as the use thereof in the agricultural sector or in related fields.

This also applies to a process for the treatment of plants which is distinguished by the application of the novel compounds soraphen C to σ of the formula I or of the corresponding novel agents.

Examples of target crops for the plant protection use disclosed in this publication, within the scope of this invention, are the following plant species: cereals (wheat, barley, rye, oats, rice, maize, sorghum and related species); beet (sugar-beet and fodder beet); pomaceous fruit, stone fruit and soft fruit (apples, pears, plums, peaches, almonds, cherries, strawberries, raspberries and blackberries); pulses (beans, lentils, peas, soya beans); oil crops (oil seed rape, mustard, poppy, olives, sunflowers, coconuts, castor, cocoa, peanuts); the gourd family (pumpkin, cucumbers, melons); fibre plants (cotton, flax, hemp, jute); citrus fruit (oranges, lemons, grapefruit, tangerines); various vegetables (spinach, lettuce, asparagus, cabbage species, carrots, onions, tomatoes, potatoes, paprika); the Lauraceae (avocado, Cinnamonium, camphor) or plants such as tobacco, nuts, coffee, pineapple, sugar cane, tea, pepper, vines, hops, the banana family and plants which yield natural rubber, as well as ornamental plants (Compositae). This enumeration does not represent any limitation.

Active ingredients of the formula I are customarily used in the form of compositions and can be applied to the area or plant to be treated either simultaneously or in succession with other active ingredients. These other active ingredients can be fertilizers, suppliers of trace elements or other preparations which influence plant growth. In this context, it is also possible to use selective herbicides as well as insecticides, fungicides, bactericides, nematicides, molluscicides or mixtures of a

plurality of these preparations, if desired together with further carriers conventionally used in the art of formulation, surfactants or other additives which assist application.

Suitable carriers and additives can be solid or liquid and correspond to the substances advantageously used in the art of formulation, for example natural or regenerated mineral substances, solvents, dispersants, wetting agents, tackifiers, thickeners, binders or fertilizers.

A preferred method of applying an active ingredient of the formula I or an agrochemical agent which contains at least one of these active ingredients, is application onto the foliage (leaf application). In this context, the frequency of application and the dosage rate depend on the infection pressure of the specific pathogen. However, the active ingredients of the formula I can also enter the plant via the soil and the root system (systemic action), by drenching the site where the plant grows with a liquid preparation, or by incorporating the substances in solid form into the soil, for example in the form of granules (soil application). These granules or a corresponding powder may also be the dry mass of the biomass produced in from the fermenter or the adsorbent resin sieved out from the fermentation broth and loaded with the active ingredients of the formula I. The compounds of the formula I can also be applied to seeds (coating), either by immersing the grains in a liquid preparation of the active ingredient or by coating them with a solid preparation.

In this context, the compounds of the formula I are employed in unaltered form or, preferably, together with the adjuvants conventionally used in the art of formulation. For this purpose, they are expediently processed in a known manner, for example to give emulsion concentrates, spreadable pastes, directly sprayable or dilutable solutions, dilute emulsions, wettable powders, soluble powders, dusts, granules or encapsulations, for example in polymeric substances. The application methods, such as spraying, misting, dusting, scattering, brushing or watering, as well as the type of the agents, are chosen to suit the intended use and the circum-

stances which prevail. Advantageous application rates are generally at around 10 g to 2 kg of active ingredient (AI) per hectare, preferably at around 50 g to 500 g of AI/ha.

The preparations, i.e. the agents containing the active ingredient of the formula I and optionally a solid or liquid additive, are prepared in a known manner.

Possible solvents are: aromatic and aliphatic hydrocarbons, for example xylene mixtures, cyclohexane or paraffins; also alcohols and glycols as well as their ethers and esters, such as ethanol, ethylene glycol, ethylene glycol monomethyl ether or ethylene glycol monoethyl ether, or acetic acid esters; ketones, such as cyclohexanone, strongly polar solvents, such as N-methyl-2-pyrrolidone, dimethyl sulfoxide or dimethylformamide, as well as epoxidized and unepoxidized vegetable oils, such as epoxidized coconut oil or soya bean oil; or water.

Solid carriers which are generally used, for example for dusting agents and dispersible powders, are ground natural minerals, such as calcite, talc, kaolin, montmorilonite or attapulgite. To improve the physical properties, it is also possible to add highly-disperse silicic acid or highly-disperse absorptive polymers. Possible adsorptive, granulated granule carriers are porous types, for example pumice, ground brick, sepiolite or bentonite, possible non-sorptive carrier materials are, for example, calcite or sand. In addition, a large range of pregranulated materials of inorganic nature, such as, in particular, dolomite, or comminuted plant residues, can be used.

Suitable surface-active compounds are non-ionogenic or cation-active and/or anion-active surfactants having good emulsifying, dispersing and wetting properties, depending on the type of the active ingredient of the formula I to be formulated. Surfactants are also understood as meaning mixtures of surfactants.

Suitable anionic surfactants can be either so-called water-soluble soaps or water-soluble synthetic surface-active compounds.

More frequently, however, so-called synthetic surfactants are used, in particular alkane sulfonates, fatty alcohol sulfates, sulfonated benzimidazole derivatives or alkyl sulfonates.

Possible non-ionic surfactants are polyglycol ether derivatives of aliphatic or cycloaliphatic alcohols, of saturated or unsaturated fatty acids and of alkylphenols, which can contain 3 to 30 glycol ether groups and 8 to 20 carbon atoms in the (aliphatic) hydrocarbon moiety and 6 to 18 carbon atoms in the alkyl moiety of the alkylphenols.

Further examples of non-ionic surfactants which may be mentioned are nonylphenol polyethoxyethanols, castor oil polyglycol ethers, polypropylene/polyethylene oxide adducts, tributylphenoxypolyethyleneethanol, polyethylene glycol and octylphenoxypolyethoxyethanol.

Further suitable substances are also fatty acid esters of polyoxyethylene sorbitan, such as polyoxyethylene sorbitan trioleate.

The surfactants which are used in the art of formulation are described, inter alia, in the following publications:

"Mc Cutcheon's Detergents and Emulsifiers Annual" MC Publishing Corp., Ridgewood New Jersey, 1980.
Sisley and Wood, "Encyclopedia of Surface Active Agents", Chemical Publishing Co., New York, 1980.

Particularly advantageous additives which promote the application and which may result in a considerable reduction in the amount applied are furthermore natural (animal or vegetable) or synthetic phospholipids from the series comprising the cephalins and lecithins such as, for example, phosphatidyl ethanolamine, phosphatidyl serin, phosphatidyl glycerol or lysolecithin.

As a rule, the agrochemical preparations contain 0.1 to 95 % of active ingredient of the formula I, 99.9 to 5 % of a solid or liquid additive and 0 to 25 % of a surfactant.

While fairly concentrated agents are preferred as a commercial good, the end consumer, as a rule, uses dilute agents.

The agents can also contain further additives, such as stabilizers, defoamers, viscosity regulators, binders, tackifiers as well as fertilizers or other active ingredients, for obtaining specific effects.

The examples which follow are intended to illustrate the invention in greater detail without imposing any limitation.

1. Preparation examples

Example H-1: Preparation of compounds of the formula I in the presence of an adsorbent resin.

The process is carried out in a fermentation volume of 1,000 litres with the addition of 0.5 % (v/v) of the adsorbent resin XAD-1180 (Rohm and Haas). The process conditions for this mixture correspond to those of Example H-2 below. Instead of the strain So ce 26 from EP-A-282,455 used here, use may also be made of one of the other 6 strains mentioned of the present invention.

After completion of the fermentation, the polymer carrier is sieved off, rinsed into a glass column, washed with 3 bed volumes of water and eluted with 4 bed volumes of methanol. The eluate is concentrated to dryness in vacuo.

Crude extract: 165 g.

The subsequent chromatographic purification follows the chart in the appended figure 1 whose individual separation steps proceed under the following conditions. The yields are specified in brackets. (h = hour).

Separation process for isolating soraphen A to Q

A'

1. Silica gel separation; column: 100 mm diameter; 45 cm long;
   Sorbent: Lichroprep* Si 100; 40-63 µm (manufacturer: Merck)
   Mobile phase: dichloromethane/acetone in steps of 98/2, 95/5, 93/7,90/10, 50/50; 2 litres per step
   5 fractions are taken.
   Fraction 3 contains soraphen A.
   Fraction 4 (25 g) is purified further.

2. Gel chromatography; column: 60 mm diameter, 100 cm long;
   Sorbent: Sephadex* LH-20 (manufacturer: Pharmacia LKB GmbH)
   Mobile phase: methanol
   5 fractions are taken.
   Fraction 3 (17 g) is purified further.

3. Reversed-phase separation; 76 mm diameter; 70 cm long;
   Sorbent: HDSIL RP-18* 18-60-60 35-70 µm (manufacturer: Labomatic)
   Mobile phase: methanol/water 40/60 linear gradient in 2 h tomethanol.
   9 fractions are taken.
   Fractions 3 (1.3 g), 4 (1.5 g), 5 (3.5 g) and 6 (1.6 g) are purified further.

4. Silica gel separation; column: 40 mm diameter; 30 cm long;
   Sorbent: Lichrosorb* Si 100; 7 µm (manufacturer: Merck)
   Mobile phase: dichloromethane/hexane 1/1 + 2 % methanol
   6 fractions are taken.
   Fractions 2 (500 mg) and 4 (88 mg) are purified further.

5. Silica gel separation; column: 40 mm diameter; 30 cm long;
   Sorbent: Lichrosorb Si 100; 7 µm (manufacturer: Merck)
   Mobile phase: dichloromethane/hexane 1/1 + 2 % methanol
   6 fractions are taken.
   Fraction 3 (500 mg) is purified further.

* Trade Mark

6. Silica gel separation; column: 35 mm diameter; 45 cm long;
Sorbent: Lichroprep Si 60; 40-63 µm (manufacturer: Merck)
Mobile phase: dichloromethane linear gradient in 1 h to dichloromethane/acetone 1/1.
6 fractions are taken.
Fractions 1 (2.4 g) and 2 (400 mg) are purified further.

7. Reversed-phase separation; column: 35 mm diameter; 45 cm long;
Sorbent: HDSIL RP-18; 18-30-60 25-40 µm (manufacturer: Labomatic)
Mobile phase: methanol/water 80/20.
9 fractions are taken.
Fraction 7 (1.4 g) is purified further.

8. Reversed-phase separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: Nucleosil 100-7 $C_{18}$; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: methanol/water 70/30.
7 fractions are taken. Fraction 3 (15 mg) is purified further.
Fraction 7 contains Soraphen N (18 mg).

9. Reversed-phase separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: Nucleosil*100-7 $C_{18}$; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: methanol/water 65/35.
7 fractions are taken.
Fraction 3 contains Soraphen H (7 mg).

10. Reversed-phase separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: HDSIL 100-$C_{18}$-10 µm (manufacturer: Labomatic)
Mobile phase: methanol/water 65/35.
4 fractions are taken.
Fraction 2 contains Soraphen D (108 mg).

11. Silica gel separation; column: 40 mm diameter; 30 cm long;
Sorbent: Lichrosorb Si 100; 7 µm (manufacturer: Merck)
Mobile phase: dichloromethane/hexane 1/1 + 1 % methanol.
9 fractions are taken.
Fractions 4 (530 mg) and 6 (280 mg) are purified further.

* Trade Mark

12. Silica gel separation; column: 40 mm diameter; 30 cm long;
Sorbent: Lichrosorb Si 100; 7 µm (manufacturer: Merck)
Mobile phase: dichloromethane/hexane 1/1 + 1 % methanol.
8 fractions are taken.
Fraction 7 (290 mg) is purified further.

13. Silica gel separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: Nucleosil Si 100-7; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: tert-butylmethyl ether/hexane 1/2 + 1 % methanol.
2 fractions are taken.
Fraction 1 (325 mg) contains Soraphen F.

14. Reversed-phase separation; column: 20.5 diameter; 25 cm long;
Sorbent: Nucleosil 100-7 $C_{18}$; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: methanol/water 68/32.
2 fractions are taken.
Fraction 1 contains Soraphen Q (3 mg).

15. Reversed-phase separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: Nucleosil 100-7 $C_{18}$; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: methanol/water 66/34.
4 fractions are taken.
Fraction 1 contains Soraphen C (21 mg).
Fraction 2 contains Soraphen B (230 mg).

16. Reversed-phase separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: Nucleosil 100-7 $C_{18}$; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: methanol/water 65/35.
10 fractions are taken.
Fraction 4 contains Soraphen J (18 mg).
Fraction 9 contains Soraphen E (96 mg).

17. Reversed-phase separation; column 20.5 mm diameter; 25 cm long;
Sorbent: Nucleosil 100-7 $C_{18}$; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: methanol/water 70/30.
10 fractions are taken.
Fraction 6 contains Soraphen M (98 mg).

Physicochemical characterization of soraphens C to Q

Soraphen C

$C_{28}H_{42}O_8$

Molecular weight 506

IR (film)

3411; 2939; 2831; 1725; 1461; 1382; 1266; 1230; 1187; 1152; 1098; 1068; 1023; 988; 975.

$^{13}$C-NMR shifts ($CDCl_3$; δ in ppm)

10.3; 11.7; 12.5; 23.0; 26.0; 29.4; 35.2; 35.6; 35.8; 46.2; 57.3; 57.6; 68.8; 72.5; 74.6; 74.9; 76.1; 83.7; 99.4; 125.0; 126.2; 126.2; 128.2; 128.6; 137.3; 141.0; 170.6.

HPLC $R_t$ = 8.6 min

Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel; flow: 1.5 ml/min; mobile phase: methanol/water 70/30; detector: UV 210 nm.

Soraphen D

$C_{27}H_{42}O_8$

Molecular weight 494

IR (film)

3411; 2937; 2831; 1733; 1461; 1432; 1382; 1359; 1328; 1268; 1208; 1195; 1096; 1050; 998; 933.

$^{13}$C-NMR shifts ($CDCl_3$; δ in ppm)

10.7; 14.4; 22.8; 24.3; 25.0; 27.5; 27.7; 31.6; 35.3; 35.6; 43.2; 57.5; 58.6; 69.1; 70.8; 71.2; 74.8; 80.7; 82.6; 97.7; 126.4; 126.4; 128.0; 128.6; 128.6; 141.0; 168.6.

HPLC $R_t$ = 7.4 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel; flow: 1.5 ml/min; mobile phase: methanol/water 70/30; detector: UV 210 nm.

Soraphen E

$C_{29}H_{46}O_9$
Molecular weight 538
IR (film)
3398; 2939; 2829; 1725; 1461; 1430; 1380; 1266; 1235; 1191; 1154; 1102; 1044; 971.

$^{13}$C-NMR shifts ($CDCl_3$; $\delta$ in ppm)
10.3; 10.5; 11.5; 23.0; 25.0; 29.5; 30.0; 35.3; 35.5; 40.2; 46.3; 56.4; 57.4; 58.0; 69.0; 71.3; 71.7; 75.0; 76.3; 80.2; 81.1; 99.9; 126.5; 126.5; 128.1; 128.6; 140.9; 170.9.

HPLC $R_t$ = 11.8 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel; flow: 1.5 ml/min; mobile phase: methanol/water 70/30; detector: UV 210 nm.

Soraphen F

$C_{29}H_{46}O_8$
Molecular weight 522
IR (film)
3394; 2937; 2827; 1729; 1710; 1461; 1382; 1326; 1314; 1266; 1253; 1232; 1189; 1154; 1104; 1075; 1048; 1021; 994; 971; 907.

$^{13}$C-NMR shifts ($CDCl_3$; $\delta$ in ppm)
10.4; 11.7; 14.1; 23.0; 24.2; 24.2; 28.3; 28.8; 33.3; 34.1; 35.2; 45.7; 57.4; 57.4; 57.9; 69.0; 70.1; 75.9; 76.3; 80.8; 82.1; 99.6; 126.6; 126.6; 128.2; 128.6; 128.6; 140.4; 171.8.

HPLC $R_t$ = 8.1 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel; flow: 1.5 ml/min; mobile phase: methanol/water 70/30; detector: UV 210 nm.

Soraphen H

$C_{28}H_{42}O_9$

Molecular weight 522

IR (film)

3444; 2935; 2867; 2833; 1723; 1459; 1409; 1382; 1334; 1270; 1230; 1179; 1156; 1104; 1069; 996; 971.

$^{13}$C-NMR shifts ($CDCl_3$; $\delta$ in ppm)

10.9; 11.6; 14.4; 23.4; 25.0; 28.6; 35.6; 36.3; 45.6; 57.7; 57.8; 67.5; 68.7; 69.2; 73.9; 73.9; 76.1; 81.7; 100.1; 122.9; 126.0; 126.0; 128.2; 128.7; 128.7; 138.0; 140.6; 171.5.

HPLC $R_t$ = 7.0 min

Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel; flow: 1.5 ml/min; mobile phase: methanol/water 65/35; detector: UV 210 nm.

Soraphen J

$C_{28}H_{44}O_8$

Molecular weight 508

IR (film)

3394; 2939; 2829; 1729; 1461; 1380; 1313; 1270; 1187; 1158; 1100; 1042; 987.

$^{13}$C-NMR shifts ($CDCl_3$; $\delta$ in ppm)

11.5; 13.8; 14.3; 22.5; 24.1; 25.6; 25.8; 27.7; 31.6; 35.2; 36.3; 49.3; 57.3; 59.4; 69.6; 70.5; 72.6; 75.7; 81.0; 84.6; 98.2; 126.3; 126.3; 128.1; 128.7; 128.7; 141.3; 172.5.

HPLC $R_t$ = 8.1 min

Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel; flow: 1.5 ml/min; mobile phase: methanol/water 70/30; detector: UV 210 nm.

Soraphen M

$C_{28}H_{44}O_9$

Molecular weight 524

IR (film)

3396; 2939; 2831; 1725; 1461; 1380; 1270; 1235; 1191; 1154; 1075; 1048; 994; 971; 898; 850.

$^{13}$C-NMR shifts ($CDCl_3$; $\delta$ in ppm)

9.0; 10.4; 11.5; 23.1; 25.3; 28.3; 29.3; 35.1; 35.7; 40.3; 46.5; 57.2; 57.9; 68.8; 68.8; 71.9; 72.9; 74.4; 76.3; 83.8; 99.6; 126.3; 126.3; 128.0; 128.5; 128.5; 141.2; 171.0.

HPLC $R_t$ = 8.5 min

Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel; flow: 1.5 ml/min; mobile phase: methanol/water 70/30; detector: UV 210 nm.

Soraphen N

$C_{29}H_{44}O_9$

Molecular weight 536

IR (film)

3444; 2966; 2939; 1731; 1461; 1380; 1309; 1268; 1224; 1164; 1098.

$^{13}$C-NMR shifts ($CDCl_3$; $\delta$ in ppm)

13.8; 13.9; 23.3; 23.4; 24.2; 26.3; 26.7; 29.5; 32.1; 36.3; 50.3; 54.5; 57.6; 68.1; 75.2; 78.4; 79.9; 105.7; 125.8; 125.8; 127.5; 128.4; 128.4.

$^1$H-NMR shifts ($CDCl_3$; $\delta$ in ppm)

0.9 d; 1.05 d; 1.24 d; 5.23 d; 5.85 dd.

HPLC $R_t$ = 13.4 min

Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel; flow: 1.5 ml/min; mobile phase: methanol/water 70/30; detector: UV 210 nm.

Soraphen Q

$C_{28}H_{44}O_8$

Molecular weight 508

IR (film)

3448; 2939; 2831; 1733; 1459; 1382; 1330; 1270; 1203; 1098; 1048; 987.

$^{13}$C-NMR shifts ($CDCl_3$; $\delta$ in ppm)

11.5; 14.5; 23.1; 24.9; 26.2; 27.1; 27.6; 31.7; 35.5; 35.9; 39.5; 50.7; 57.4; 58.7; 69.5; 71.3; 71.6; 74.6; 79.4; 81.9; 102.2; 126.6; 126.6; 127.9; 128.4; 137.9; 168.0.

HPLC $R_t$ = 7.7 min

Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel; flow: 1.5 ml/min; mobile phase: methanol/water 70/30; detector: UV 210 nm.

Example H-2: Preparation of the compounds of the formula I

a) Preculture:

The preculture is cultured in 2-litre flasks each containing 500 ml of a culture medium (containing 0.1 % peptone from casein, 0.5 % glucose, 0.05 % $CaCl_2.H_2O$, 0.05 % $MgSO_4.7H_2O$) at pH 7.4 without buffer (or with 50 mM HEPES buffer[2]) at 160 rpm and 30°C on a vibrating table. The most favourable time for transfer to the fermenter culture is reached after 2-3 days (upper log phase). Instead of the strain So ce 192 used here, one of the other 5 strains of the present invention or the strain So ce 26 in accordance with EP-A-282,455 may also be used.

b) Fermentation:

A 70-litre fermenter supplied by the Giovanola Frères company, Monthey, Switzerland, containing 60 litres of the same culture medium is inoculated with 10 litres of preculture. The fermentation is carried out at 30-32°C. The stirring speed is 500 rpm, the aeration rate 0.12 litre per litre of medium and hour. The duration of fermentation is 7-14 days. It is ensured that the pH does not drop below 7.0, in particular below 6.2. The macrocyclic compounds of the formula I formed are partly in the cul-

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2 HEPES is the K and/or Na salt of 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid

ture supernatant, partly in the cells. They may be extracted from the cells with alcohols or ketones (for example, acetone), and from the culture supernatant with ethyl acetate or butyl acetate. The fermentation broth is then shaken out, for example five times using 2 litres of ethyl acetate in each case for 5 minutes in each case. These combined extracts are washed twice with water and concentrated in vacuo. The residual dark oil can be separated by the method shown in Figure 2.

It is more advantageous, however, to remove the compounds of the formula I from the fermentation volume with adsorbent resin after completion of the fermentation. For this purpose, 0.5 % v/v of fine-grained resin (for example XAD-1180 or XAD-16) is added to the fermenter broth and stirred for 4 hours, after which the compounds of the formula I are completely bound to the resin. After separating off the fermenter broth by sieving off, the resin is rinsed into a glass column, washed with 3 bed volumes of water and eluted with 4 bed volumes of methanol. The eluate is concentrated to dryness in vacuo. Such an eluate or such a crude extract can be formulated with suitable dispersants and/or extenders to produce commercially usable plant protection agents, but the crude extract can also be separated to isolate the individual soraphens A to σ as shown below and in the attached Figure 2. The separation process as shown hereunder was carried out with 65 g of crude extract obtained from 400 litres of fermenter both.

Chromatographic purification (see: separation process II for isolating soraphens)

1 Silica gel separation; column: 200 mm diameter; 200 mm long;
Sorbent: Lichroprep Si 100; 25-40 µm (manufacturer: Merck)
Mobile phase: gradient in stages; 1. dichloromethane; 2.-9. dichloromethane/acetone 98/2, 96/4, 95/5, 90/10, 85/15, 80/20, 50/50, 25/75;
10.-11. dichloromethane/methanol 75/25, 50/50;
14 fractions are taken.
Fractions 2, 3, 5, 6, 7, 8, 9, 10, 11 are purified further.

2 Gel chromatography; column: 60 mm diameter; 100 cm long;
Sorbent: Sephadex LH-20 (manufacturer: Pharmacia LKB GmbH)
Mobile phase: methanol
7 fractions are taken.
Fraction 3 contains soraphen A (2.5 g).

3 Gel chromatography; column: 30 mm diameter; 100 cm long;
Sorbent: Sephadex LH-20 (manufacturer: Pharmacia LKB GmbH)
Mobile phase: methanol
5 fractions are taken.
Fraction 2 contains soraphen C (6.6 g).

4 Gel chromatography; column: 30 mm diameter; 100 cm long;
Sorbent: Sephadex LH-20 (manufacturer: Pharmacia LKB GmbH)
Mobile phase: methanol
6 fractions are taken.
Fraction 2 (1 g) is purified further.

5 Reversed-phase separation; column: 37 mm diameter; 34 cm long;
Sorbent: HDSIL RP-18; 18-20-60 15-25 µm (manufacturer: Labomatic)
Mobile phase: methanol/water 72/28.
9 fractions are taken.
Fraction 7 contains soraphen V (1.1 g).

6 Gel chromatography; column: 30 mm diameter; 100 cm long;
Sorbent: Sephadex LH-20 (manufacturer: Pharmacia LKB GmbH)
Mobile phase: methanol
4 fractions are taken.
Fraction 2 (1.1 g) is purified further.

7 Gel chromatography; column: 30 mm diameter; 100 cm long;
Sorbent: Sephadex LH-20 (manufacturer: Pharmacia LKB GmbH)
Mobile phase: methanol
6 fractions are taken.
Fractions 2 (0.54 g) and 3 (0.18 g) are purified further.

8 Gel chromatography; column: 30 mm diameter; 100 cm long;
Sorbent: Sephadex LH-20 (manufacturer: Pharmacia LKB GmbH)
Mobile phase: methanol
6 fractions are taken.
Fractions 2 (0.3 g) and 3 (1.3 g) are purified further.

9 Gel chromatography; column: 60 mm diameter; 100 cm long;
Sorbent: Sephadex LH-20 (manufacturer: Pharmacia LKB GmbH)
Mobile phase: methanol
7 fractions are taken.
Fraction 2 (3.3 g) is purified further.

10 Gel chromatography; column: 60 mm diameter; 100 cm long;
Sorbent: Sephadex LH-20 (manufacturer: Pharmacia LKB GmbH)
Mobile phase: methanol
6 fractions are taken.
Fraction 2 (6.1 g) is purified further.

11 Reversed-phase separation; column: 37 mm diameter; 34 cm long;
Sorbent: HDSIL RP-18; 18-20-60 15-25 µm (manufacturer: Labomatic)
Mobile phase: methanol/water 75/25.
8 fractions are taken.
Fraction 5 contains soraphen D (40 mg).
Fraction 7 contains soraphen B (0.5 g).

12 Reversed-phase separation; column: 35 mm diameter; 45 cm long;
Sorbent: HDSIL RP-18; 18-30-60 25-40 µm (manufacturer: Labomatic)
Mobile phase: methanol/water 80/20.
10 fractions are taken.
Fraction 8 contains soraphen E (30 mg).
Fraction 4 is purified further.

13 Reversed-phase separation; column: 37 mm diameter; 34 cm long;
Sorbent: HDSIL RP-18; 18-20-60 15-25 µm (manufacturer: Labomatic)
Mobile phase: methanol/water 75/25.
10 fractions are taken.
Fraction 6 (80 mg) is purified further.
Fraction 8 (20 mg) is purified further.

14 Reversed-phase separation; column: 37 mm diameter; 34 cm long;
Sorbent: HDSIL RP-18; 18-20-60 15-25 µm (manufacturer: Labomatic)
Mobile phase: methanol/water 75/25.
11 fractions are taken.
Fraction 5 contains soraphen X (16 mg).

15 Reversed-phase separation; column: 35 mm diameter; 45 cm long;
Sorbent: HDSIL RP-18; 18-30-60 25-40 µm (manufacturer: Labomatic)
Mobile phase: methanol/water 70/30 linear gradient in 2 h to methanol.
8 fractions are taken.
Fraction 5 contains soraphen U (150 mg).

16 Reversed-phase separation; column: 35 mm diameter; 45 cm long;
Sorbent: HDSIL RP-18; 18-30-60 25-40 µm (manufacturer: Labomatic)
Mobile phase: methanol/water 75/25 linear gradient in 1 h to 90/10.
9 fractions are taken.
Fraction 8 contains soraphen M (0.93 g).
Fraction 7 (250 mg) is purified further.

17 Reversed-phase separation; column: 37 mm diameter; 34 cm long;
Sorbent: HDSIL RP-18; 18-20-60 15-25 µm (manufacturer: Labomatic)
Mobile phase: methanol/water 70/30.
13 fractions are taken.
Fraction 4 (105 mg) is purified further.
Fraction 6 contains soraphen R (20 mg).
Fraction 7 contains soraphen S (490 mg).
Fraction 8 contains soraphen T (80 mg).
Fraction 9 (80 mg) is purified further.

18 Reversed-phase separation; column: 37 mm diameter; 34 cm long;
Sorbent: HDSIL RP-18; 18-20-60 15-25 µm (manufacturer: Labomatic)
Mobile phase: methanol/water 70/30.
14 fractions are taken.
Fraction 7 (700 mg) is purified further.
Fraction 8 (570 mg) is purified further.
Fraction 9 contains soraphen δ (120 mg).

19 Reversed-phase separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: RP-18 Nucleosil 100-7 $C_{18}$; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: methanol/water 67/33.
6 fractions are taken.
Fraction 5 contains soraphen γ (11 mg).

20 Silica gel separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: Si 100 Nucleosil 100-7; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: tert-butyl methyl ether/n-hexane/+methanol 1/2/+ 1 %
3 fractions are taken.
Fraction 2 contains soraphen ν (8 mg).

21 Silica gel separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: Si 100 Nucleosil 100-7; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: tert-butyl methyl ether/n-hexane/+methanol 1/2/+ 1 %
2 fractions are taken.
Fraction 2 contains soraphen β (8 mg).

22 Silica gel separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: Si 100 Nucleosil 100-7; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: tert-butyl methyl ether/n-hexane/+methanol 1/2/+ 5 %
7 fractions are taken.
Fraction 1 contains soraphen Y (64 mg).
Fraction 4 contains soraphen o (5 mg).
Fraction 6 contains soraphen ξ (7 mg).

23 Reversed-phase separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: RP-18 Nucleosil 100-7 $C_{18}$; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: methanol/water 60/40.
6 fractions are taken.
Fraction 2 (35 mg) is purified further.

24 Silica gel separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: Si 100 Nucleosil 100-7; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: tert-butyl methyl ether/n-hexane/+methanol 1/2/+ 10 %
5 fractions are taken.
Fraction 2 contains soraphen σ (20 mg).

25 Reversed-phase separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: RP-18 Nucleosil 100-7 $C_{18}$; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: methanol/water 60/40.
9 fractions are taken.
Fraction 4 (194 mg) is purified further.
Fraction 5 (130 mg) is purified further.
Fraction 6 (50 mg) is purified further.

26 Silica gel separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: Si 100 Nucleosil 100-7; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: tert-butyl methyl ether/n-hexane/+ methanol 1/2/+ 5 %
9 fractions are taken.
Fraction 4 contains soraphen ρ (45 mg).

27 Silica gel separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: Si 100 Nucleosil 100-7; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: tert-butyl methyl ether/n-hexane/+ methanol 1/2/+ 10 %
4 fractions are taken.
Fraction 2 contains soraphen ζ (26 mg).

28 Silica gel separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: Si 100 Nucleosil 100-7; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: tert-butyl methyl ether/n-hexane/+ methanol 1/2/+ 5 %
4 fractions are taken.
Fraction 2 contains soraphen µ (18 mg).
Fraction 3 contains soraphen η (69 mg).

29 Silica gel separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: Si 100 Nucleosil 100-7; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: tert-butyl methyl ether/n-hexane/+ methanol 1/2/+ 5 %
5 fractions are taken.
Fraction 4 contains soraphen κ (5.5 mg).

30 Silica gel separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: Si 100 Nucleosil 100-7; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: tert-butyl methyl ether/n-hexane/+ methanol 1/2/+ 5 %
4 fractions are taken.
Fraction 4 (10 mg) is purified further.

31 Silica gel separation; column: 20.5 mm diameter; 25 cm long;
Sorbent: Si 100 Nucleosil 100-7; 7 µm (manufacturer: Macherey Nagel)
Mobile phase: tert-butyl methyl ether/n-hexane/+ methanol 1/2/+ 5 %
2 fractions are taken.
Fraction 1 contains soraphen π (2.2 mg).
Fraction 2 contains soraphen Z (6.5 mg).

Physicochemical characterization of the additionally obtained soraphens R to σ

Soraphen V

$C_{28}H_{42}O_8$

Molecular weight 506

IR (film, ν in $cm^{-1}$)

3394; 2942; 2900; 2829; 1723; 1461; 1380; 1270; 1233; 1189; 1068; 988; 898; 851.

$^{13}$C-NMR shifts ($CDCl_3$; δ in ppm)
10.3; 11.7; 12.6; 23.2; 26.0; 32.7; 35.3; 35.6; 36.2; 46.3; 55.8; 57.3; 68.9; 72.5; 72.9; 74.6; 76.3; 84.5; 99.5; 122.1; 126.2; 126.2; 128.1; 128.5; 128.5; 139.9; 141.2; 170.9.

HPLC $R_t$ = 9.2 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen X

$C_{28}H_{42}O_9$
Molecular weight 522
IR (film, ν in $cm^{-1}$)
3404; 2941; 1716; 1598; 1461; 1380; 1272; 1233; 1189; 1156; 1100; 1069; 988.

$^{13}$C-NMR shifts ($CDCl_3$; δ in ppm)
10.3; 11.7; 12.5; 23.1; 25.9; 29.4; 35.2; 35.6; 35.7; 46.2; 57.3; 68.9; 72.5; 74.5; 75.0; 76.1; 83.8; 99.5; 113.4; 115.2; 118.0; 124.9; 129.8; 137.4; 142.7; 156.2; 170.9.

HPLC $R_t$ = 4.4 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen U

$C_{29}H_{44}O_9$
Molecular weight 536
IR (film, ν in $cm^{-1}$)
3386; 3361; 2977; 2939; 2892; 2823; 1698; 1461; 1380; 1268; 1185; 1152; 1096; 1064; 1023; 975; 900; 840.

$^{13}$C-NMR shifts ($CDCl_3$; δ in ppm)
10.1; 11.4; 12.2; 31.8; 32.9; 35.2; 35.2; 38.6; 46.3; 56.0; 57.2; 57.9; 64.2; 68.7; 72.2; 73.3; 76.2; 80.0; 84.5; 99.3; 122.2; 126.1; 126.1; 127.9; 128.4; 128.4; 140.6; 141.4; 171.7.

HPLC $R_t$ = 5.2 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen R

$C_{26}H_{40}O_8$
Molecular weight 480
IR (film, ν in $cm^{-1}$)
3404; 2937; 1731; 1459; 1430; 1384; 1355; 1330; 1270; 1212; 1110; 1083; 1033; 988.

$^{13}$C-NMR shifts ($CDCl_3$; δ in ppm)
10.7; 14.5; 22.7; 23.3; 24.8; 27.9; 30.9; 31.8; 35.5; 35.3; 43.4; 58.6; 68.9; 71.0; 72.9; 73.3; 74.6; 80.5; 97.6; 126.5; 126.5; 128.0; 128.6; 128.6; 141.1; 168.8.

HPLC $R_t$ = 5.1 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen S

$C_{27}H_{40}O_8$
Molecular weight 492
IR (film, ν in $cm^{-1}$)
3431; 2941; 1720; 1604; 1459; 1426; 1380; 1264; 1187; 1150; 1104; 1062; 977.

$^{13}$C-NMR shifts ($CDCl_3$; δ in ppm)
10.5; 11.9; 12.7; 24.9; 26.2; 35.5; 36.2; 36.3; 37.9; 47.5; 57.6; 69.5; 73.5; 74.1; 75.8; 76.3; 77.9; 100.6; 125.1; 127.1; 127.1; 128.7; 129.4; 129.4; 138.8; 143.3; 173.8.

HPLC $R_t$ = 5.9 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen T
$C_{27}H_{42}O_8$
Molecular weight 494
IR (film, ν in $cm^{-1}$)
3427; 2941; 1720; 1461; 1382; 1268; 1191; 1154; 1106; 1068; 994; 971.

$^{13}$C-NMR shifts ($CDCl_3$; δ in ppm)
10.4; 11.7; 14.2; 22.7; 24.8; 25.3; 28.3; 30.6; 32.8; 34.6; 35.2; 45.8; 57.3; 69.1; 69.8; 73.0; 73.3; 76.0; 76.4; 99.6; 126.5; 126.5; 128.1; 128.5; 128.5; 140.6; 171.9.

HPLC $R_t$ = 6.5 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen δ (delta)
$C_{27}H_{42}O_8$
Molecular weight 506
IR (film, ν in $cm^{-1}$)
3440; 3311; 2937; 1731; 1600; 1461; 1380; 1340; 1185; 1096; 988; 850.

$^{13}$C-NMR shifts ($CDCl_3$; δ in ppm)
10.2; 12.0; 12.7; 23.2; 25.6; 29.6; 34.9; 36.0; 37.3; 45.3; 56.2; 57.6; 70.3; 72.1; 72.6; 74.3; 74.9; 84.2; 99.5; 124.9; 126.3; 126.3; 128.0; 128.6; 128.6; 131.6; 141.2; 171.2.

HPLC $R_t$ = 7.1 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen γ (gamma)
$C_{28}H_{42}O_9$
Molecular weight 522
IR (film, ν in $cm^{-1}$)
3398; 2939; 1723; 1461; 1382; 1264; 1189; 1152; 1102; 1066; 975; 894.

$^{13}$C-NMR shifts ($CDCl_3$; δ in ppm)
8.6; 10.3; 11.5; 23.6; 25.8; 28.8; 33.9; 35.4; 36.3; 46.6; 54.7; 57.3;
58.1; 59.2; 68.8; 69.3; 73.5; 74.3; 76.2; 83.5; 99.7; 126.1; 126.1;
128.0; 128.6; 141.4; 170.4.

HPLC $R_t$ = 8.4 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen ν (nu)
$C_{28}H_{42}O_9$
Molecular weight 522
IR (film, ν in $cm^{-1}$)
3402; 2971; 2935; 2827; 1733; 1459; 1367; 1181; 1095; 1050; 990; 860.

$^{13}$C-NMR shifts ($CDCl_3$; δ in ppm)
8.7; 10.3; 11.5; 26.3; 33.4; 35.2; 40.2; 40.8; 46.2; 57.4; 58.1; 67.8;
68.9; 71.5; 73.5; 76.3; 78.9; 82.6; 99.4; 125.9; 125.9; 128.3; 128.7;
128.7; 128.8; 129.5; 140.7; 170.3.

HPLC $R_t$ = 8.5 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen β (beta)
$C_{29}H_{44}O_8$
Molecular weight 520
IR (film, ν in $cm^{-1}$)
3408; 2971; 2935; 2825; 1733; 1459; 1365; 1343; 1181; 1095; 1050; 990; 958; 842; 738.

$^{13}C$-NMR shifts ($CDCl_3$; δ in ppm)
10.1; 12.1; 12.6; 23.4; 25.5; 30.4; 35.0; 35.9; 37.2; 45.3; 56.2; 56.3; 57.9; 70.3; 72.1; 72.7; 74.2; 83.1; 84.8; 99.4; 122.6; 126.3; 126.3; 128.0; 128.6; 128.6; 140.0; 141.3; 171.3.

HPLC $R_t$ = 16.8 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen Y
$C_{27}H_{40}O_9$
Molecular weight 508
IR (film, ν in $cm^{-1}$)
3436; 2937; 1721; 1459; 1376; 1268; 1199; 1114; 1048; 988; 961.

$^{13}C$-NMR shifts ($CDCl_3$; δ in ppm)
10.8; 11.2; 18.8; 23.0; 23.5; 31.7; 33.4; 36.2; 40.6; 41.1; 51.1; 61.1; 70.8; 74.6; 76.4; 77.1; 78.7; 86.4; 99.9; 125.5; 125.5; 127.4; 128.4; 128.4; 142.5; 171.1; 212.4.

HPLC $R_t$ = 6.1 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen o (omicron)
$C_{29}H_{44}O_9$
Molecular weight 536
IR (film, $\nu$ in $cm^{-1}$)
3402; 3361; 2933; 2894; 1708; 1457; 1360; 1269; 1187; 1150; 1096; 1062;
975; 898; 838; 765.

$^{13}C$-NMR shifts ($CDCl_3$; $\delta$ in ppm)
10.4; 11.6; 12.6; 31.7; 33.4; 35.3; 35.7; 38.7; 46.3; 56.3; 57.4; 58.3;
65.7; 69.0; 72.3; 74.2; 76.2; 80.0; 84.8; 99.5; 122.9; 126.3; 126.3;
128.2; 128.6; 128.6; 140.1; 140.7; 170.8.

HPLC $R_t$ = 4.8 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen ξ (xi)
$C_{27}H_{40}O_8$
Molecular weight 492
IR (film, $\nu$ in $cm^{-1}$)
3402; 2969; 2933; 1731; 1459; 1367; 1179; 1095; 1050; 990.

$^{13}C$-NMR shifts ($CDCl_3$; $\delta$ in ppm)
10.4; 11.7; 12.5; 20.1; 26.2; 31.8; 35.2; 35.7; 36.2; 46.4; 57.4;
68.9; 72.3; 73.5; 75.0; 76.1; 76.3; 99.6; 126.2; 126.2; 127.0; 128.1;
128.6; 128.6; 138.3; 141.2; 170.7.

HPLC $R_t$ = 4.1 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen σ (sigma)
$C_{29}H_{44}O_9$
Molecular weight 536
IR (film, ν in $cm^{-1}$)
3398; 3363; 3301; 2971; 2933; 2894; 1702; 1453; 1378; 1262; 1167; 1150; 1100; 979; 838.

$^{13}C$-NMR shifts ($CDCl_3$; δ in ppm)
10.3; 11.5; 12.4; 31.6; 34.2; 35.0; 35.5; 38.9; 46.2; 56.1; 57.5; 58.5; 64.3; 69.1; 73.1; 76.1; 77.2; 79.8; 85.2; 99.7; 121.8; 126.0; 126.0; 128.0; 128.5; 128.5; 141.9; 142.0; 171.4.

HPLC $R_t$ = 6.2 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen ρ (Rho)
$C_{27}H_{42}O_9$
Molecular weight 510
IR (film, ν in $cm^{-1}$)
3398; 3363; 3301; 2971; 2933; 2894; 1700; 1453; 1378; 1262; 1187; 1150; 1100; 1064; 979; 898; 838; 765.

$^{13}C$-NMR shifts ($CDCl_3$; δ in ppm)
8.7; 10.3; 11.4; 23.1; 25.5; 26.4; 32.3; 35.0; 36.1; 40.5; 46.6; 57.2; 68.5; 68.8; 71.8; 73.1; 73.7; 74.3; 76.4; 99.7; 126.3; 127.9; 128.5; 128.5; 141.4; 171.4.

HPLC $R_t$ = 4.7 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen ζ (zeta)
$C_{28}H_{42}O_9$
Molecular weight 522
IR (film, ν in $cm^{-1}$)
3398; 2937; 1718; 1457; 1378; 1332; 1272; 1203; 1102; 978.

$^{13}$C-NMR shifts ($CDCl_3$; δ in ppm)
11.8; 21.9; 25.9; 25.9; 28.7; 36.3; 39.9; 43.0; 45.7; 56.7; 57.4; 58.6;
70.6; 75.5; 76.2; 78.2; 78.2; 84.3; 98.9; 125.9; 125.9; 128.0; 128.4;
128.7; 128.7; 133.1; 141.2; 171.6.

HPLC $R_t$ = 3.5 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen μ (mu)
$C_{28}H_{44}O_9$
Molecular weight 524
IR (film, ν in $cm^{-1}$)
3423; 2939; 1725; 1459; 1380; 1264; 1191; 1154; 1102; 1052; 992; 971.

$^{13}$C-NMR shifts ($CDCl_3$; δ in ppm)
10.4; 11.7; 14.1; 21.3; 27.7; 28.2; 30.9; 32.9; 34.1; 35.2; 45.6; 57.4;
60.7; 69.1; 70.0; 70.4; 72.1; 76.2; 76.3; 85.4; 99.6; 126.6; 126.6;
128.2; 128.6; 128.6; 140.2; 172.0.

HPLC $R_t$ = 4.7 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen η (eta)
$C_{28}H_{42}O_9$
Molecular weight 522
IR (film, ν in $cm^{-1}$)
3411; 2935; 1718; 1459; 1382; 1266; 1189; 1152; 1104; 1066; 981.

$^{13}C$-NMR shifts ($CDCl_3$; δ in ppm)
10.2; 11.4; 12.3; 31.6; 32.9; 35.1; 35.4; 38.1; 46.2; 57.2; 57.7; 64.0; 68.8; 72.2; 73.7; 74.7; 76.2; 81.2; 99.3; 124.8; 126.1; 126.1; 127.9; 128.4; 128.4; 137.8; 141.1; 171.4.

HPLC $R_t$ = 4.6 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen κ (kappa)
$C_{27}H_{40}O_8$
Molecular weight 492
IR (film, ν in $cm^{-1}$)
3411; 2935; 1731; 1461; 1382; 1347; 1270; 1189; 1104; 1048; 990.

$^{13}C$-NMR shifts ($CDCl^3$; δ in ppm)
10.1; 12.1; 12.8; 23.4; 25.4; 33.5; 35.0; 36.1; 37.2; 45.4; 56.1; 70.3; 72.0; 72.7; 74.2; 74.2; 75.6; 99.4; 124.1; 126.2; 126.2; 127.9; 128.5; 128.5; 138.6; 141.3; 171.5.

HPLC $R_t$ = 4.7 min
Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;
detector: UV 210 nm.

Soraphen π (pi)

$C_{27}H_{40}O_8$

Molecular weight 492

IR (film, ν in $cm^{-1}$)

3396; 3363; 3299; 2969; 2933; 2694; 1702; 1451; 1378; 1262; 1167; 1150; 1100; 979.

$^{13}C$-NMR shifts ($CDCl_3$; δd in ppm)

10.3; 12.3; 14.6; 23.4; 25.0; 32.2; 35.6; 36.0; 36.8; 50.4; 55.9; 70.2; 71.2; 73.9; 75.0; 75.5; 98.3; 125.2; 126.2; 126.2; 128.1; 128.6; 128.6; 137.8; 141.0; 171.5.

HPLC $R_t$ = 4.9 min

Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;

flow: 1.5 ml/min; mobile phase: methanol/water 70/30;

detector: UV 210 nm.

Soraphen Z

$C_{28}H_{42}O_9$

Molecular weight 522

IR (film, ν in $cm^{-1}$)

3405; 2937; 1718; 1459; 1380; 1266; 1189; 1152; 1104; 1069; 987; 902; 850; 813; 738.

$^{13}C$-NMR shifts ($CDCl_3$; δ in ppm)

10.5; 11.7; 12.6; 31.8; 33.5; 35.3; 35.7; 41.1; 46.3; 56.0; 57.3; 66.0; 69.0. 70.5; 72.5; 74.3; 76.2; 84.3; 99.5; 122.4; 126.2; 126.2; 128.2; 128.6; 128.6; 140.4; 140.9; 170.9.

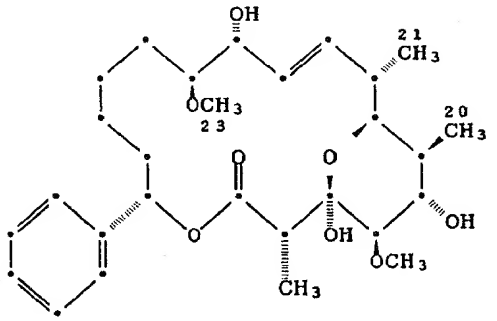
HPLC $R_t$ = 7.7 min

Column: 4 x 250 mm Nucleosil 100-7 $C_{18}$, Macherey Nagel;

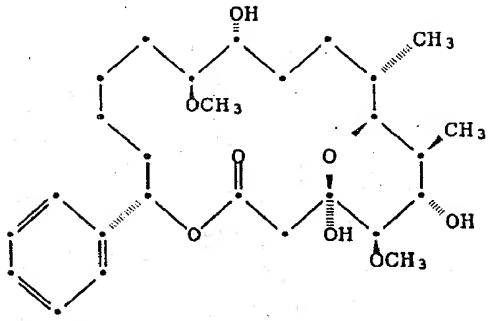
flow: 1.5 ml/min; mobile phase: methanol/water 70/30;

detector: UV 210 nm.

On the basis of the X-ray structural analysis of soraphen A and the data determined above, the following steric structures IC' to IQ' are assumed for the soraphens C to Q:



(IC')



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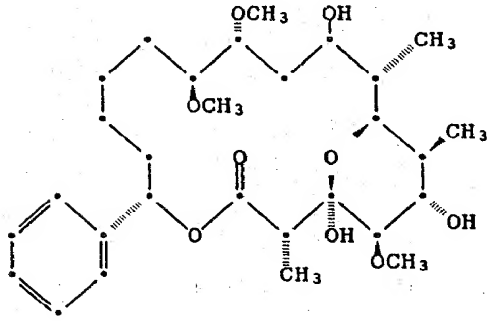

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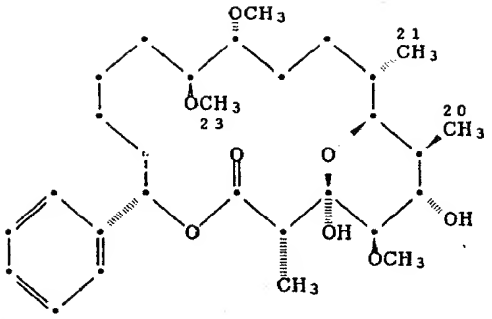

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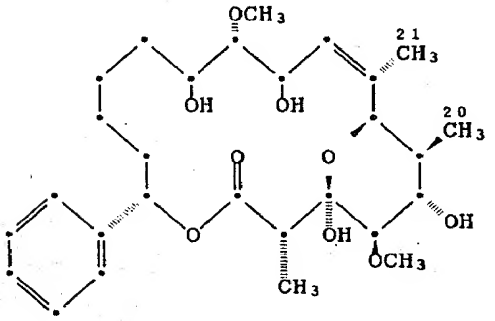

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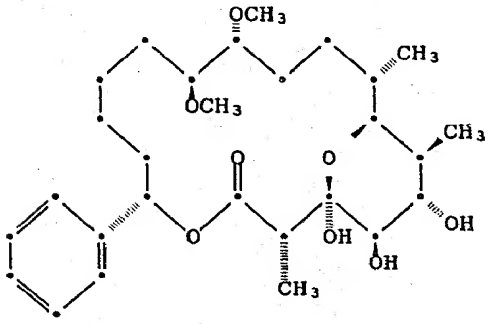

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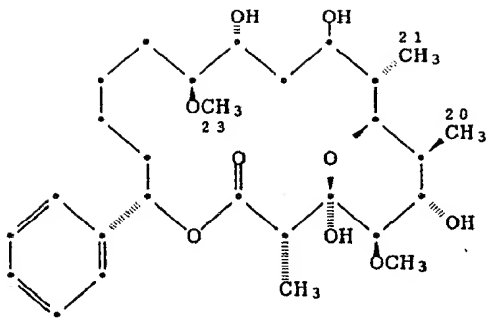

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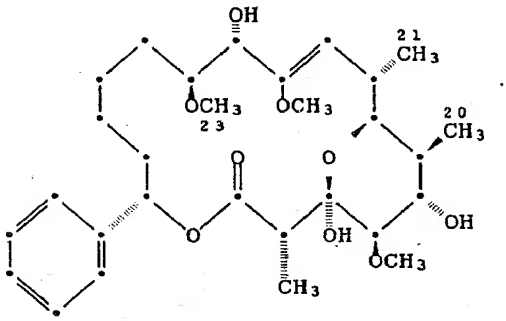

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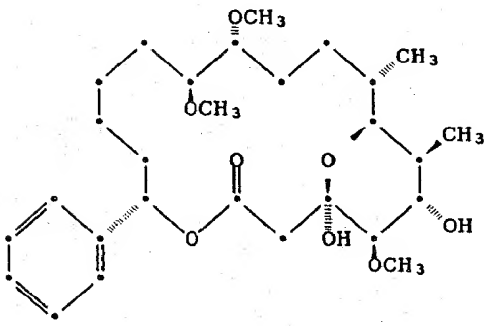
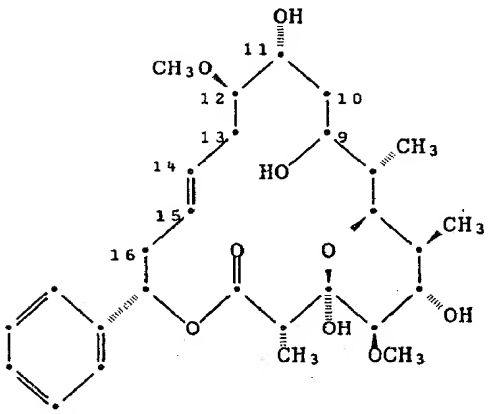


(IQ')

Similar configurations are assumed for the remaining compounds IR', IS', IT', IU', IV', IX', IY', IZ', Iβ', Iγ', Iδ', Iζ', Iη', Iκ', Iμ', Iξ' and Iρ'.

The following configuration is probable for soraphen Iν':

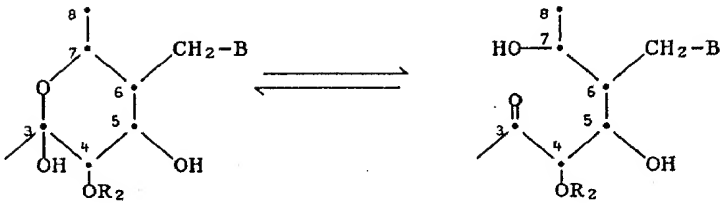


(Iν')

The configuration has not been established with certainty for the soraphens o, π and σ. In addition, it is evident that individual soraphens are epimeric with respect to one another. Soraphen δ is epimeric with respect to soraphen C; the soraphens κ and ξ are epimeric with respect to one another and to soraphen S, and the same applies to the soraphens U,o and σ. The measured physical values determined for a particular compound of the formula I which, as the person skilled in the art knows, sometimes do not make possible any strict assignment to a particular configuration, are in each case binding.

As mentioned, the invention comprises all the stereoisomeric forms of the individual listed compounds of the formula I and also their preparation and their use for combating or preventing plant diseases. Furthermore, the invention includes, however, in particular also the compounds IC' to Iσ', for which the configurations shown above are assumed, on the basis of their physicochemical data.

It should be borne in mind that the macrocyclic soraphens of the formula I are normally in the hemiacetal form indicated, but this form can undergo a reversible ring fission in accordance with the scheme:



Depending on the methods of preparation or of working up the soraphens are produced in one form or the other or as a mixture of the two forms, depending on the pH and the solvent. Characteristic of the ring fission is the shift of the $^{13}$C-NMR signal in the 3-position and that of the $^{1}$H-NMR signals in other particular positions. In the case of soraphen A, for example, the following changes are observed. $^{13}$C-NMR($CDCl^{3}$, $\delta$ in ppm) 99.5 -> 203.1(3-C). $^{1}$H-NMR($CDCl_3$, $\delta$ in ppm): 3.14 -> 3.72 (2-H); 3.18 -> 4.5(4-H); 3.83 -> 31.6 (7-H); 5.86 -> 5.7 (17-H). Similar shifts are also observed in the case of the soraphens B to $\rho$. The formula I of the present invention comprises in principle both the 3-hemiacetal form preferred at low pHs and also the fissioned 3-keto-7-hydroxyl form.

2. Formulation examples for one or more of the active ingredients of the formula I (% = percent by weight)

In the following, an active ingredient is one of the soraphens in pure form or a plurality of the soraphens C to $\sigma$ or an eluate, obtained from the microbiological preparation in liquid form or as a dry residue which contains the unseparated total amount of the soraphens.

| 2.1 Emulsion concentrates | a) | b) | c) |
|---|---|---|---|
| Active ingredient | 25 % | 40 % | 50 % |
| Ca dodecylbenzenesulfonate | 5 % | 8 % | 6 % |
| Castor oil polyethylene glycol ether (36 mol of ethylene oxide) | 5 % | - | - |
| Tributylphenol polyethylene glycol ether (30 mol of ethylene oxide) | - | 12 % | 4 % |
| Cyclohexanone | - | 15 % | 20 % |
| Xylene mixture | 65 % | 25 % | 20 % |

Emulsions of any desired concentration can be prepared from such concentrates by dilution with water.

| 2.2 Solutions | a) | b) | c) | d) |
|---|---|---|---|---|
| Active ingredient | 80 % | 10 % | 5 % | 95 % |
| Ethylene glycol monomethyl ether | 20 % | - | - | - |
| Polyethylene glycol MW 400 | - | 70 % | - | - |
| N-Methyl-2-pyrrolidone | - | 20 % | - | - |
| Epoxidized coconut oil | - | - | 1 % | 5 % |
| Petroleum ether (boiling range 160-190°C) | - | - | 94 % | - |

(MW = molecular weight)

The solutions are suitable for application in the form of microdroplets.

| 2.3 Granules | a) | b) |
|---|---|---|
| Active ingredient | 5 % | 10 % |
| Kaolin | 94 % | - |
| Highly-disperse silicic acid | 1 % | - |
| Attapulgite | - | 90 % |

The active ingredient is dissolved in methylene chloride, the solution is sprayed onto the carrier, and the solvent is then evaporated in vacuo.

| 2.4 Dusts | a) | b) |
|---|---|---|
| Active ingredient | 2 % | 5 % |
| Highly-disperse silicic acid | 1 % | 5 % |
| Talc | 97 % | - |
| Kaolin | - | 90 % |

Intimate mixing of the carrier substances with the active ingredient gives ready-to-use dusts. With the further addition of the three carrier substances, these dusts can be ground to give dusts ready for application containing 0.001 % of active ingredient.

| 2.5 Wettable powders | a) | b) | c) |
|---|---|---|---|
| Active ingredient | 25 % | 50 % | 75 % |
| Na ligninsulfonate | 5 % | 5 % | - % |
| Na laurylsulfate | 3 % | - | 5 % |
| Na diisobutylnaphthalenesulfonate | - | 6 % | 10 % |
| Octylphenol polyethylene glycol ether (7-8 mol of ethylene oxide) | - | 2 % | - % |
| Highly-disperse silicic acid | 5 % | 10 % | 10 % |
| Kaolin | 62 % | 27 % | - |

The active ingredient is thoroughly mixed with the additives, and the mixture is thoroughly ground in a suitable mill. This gives wettable powders which can be diluted with water to give suspensions of any desired concentration.

2.6 Coated granules

| | |
|---|---|
| Active ingredient | 3 % |
| Polyethylene glycol (MW 200) | 3 % |
| Kaolin | 94 % |

(MW = molecular weight)

In a mixer, the kaolin moistened with polyethylene glycol is evenly coated with the finely ground active ingredient. In this manner, dust-free coated granules are obtained.

2.7 Suspension concentrate

| | |
|---|---|
| Active ingredient | 40% |
| Ethylene glycol | 10% |
| Nonylphenol polyethylene glycol ether (15 mol of ethylene oxide) | 6% |
| Na ligninsulfonate | 10% |
| Carboxymethylcellulose | 1% |
| 37% aqueous formaldehyde solution | 0.2% |
| Silicone oil in the form of a 75% aqueous emulsion | 0.8% |
| Water | 32% |

The finely ground active ingredient is intimately mixed with the additives. This gives a suspension concentrate, from which suspensions of any desired concentration can be prepared by dilution with water.

2.8 Biomass concentrate

The biomass yielded by the production culture is dried, ground and mixed with ethylene glycol monomethyl ether in a weight ratio of 80:20. Such a concentrate can be diluted as desired with water to produce spraying suspensions.

3. Biological examples on plants

(In the following "active ingredient" denotes one of the soraphens C to σ according to the present invention).

Example 3.1: Action against Puccinia graminis on wheat

a) Residual-protective action

6 days after sowing, wheat plants are sprayed with a spray liquor (0.02 % of active ingredient) prepared from a wettable powder of the active ingredient. After 24 hours, the treated plants are infected with a uredospore suspension of the fungus. After incubation for 48 hours at 95-100 % relative atmospheric humidity and about 20°C, the infected plants are placed in a greenhouse at about 22°C. The development of rust pustules is assessed 12 days after infection.

b) Systemic action

5 days after sowing, a spray liquor (0.002 % of active ingredient relative to the soil volume) prepared from a wettable powder of the active ingredient, is poured on to wheat plants. After 48 hours, the treated plants are infected with a uredospore suspension of the fungus. After incubation for 48 hours at 95-100 % relative atmospheric humidity and about 20°C, the infected plants are placed in a greenhouse at about 22°C. The development of rust pustules is assessed 12 days after the infection.

In both experiments, fungal infestation was inhibited completely by the active ingredient.

In contrast, untreated, infected control plants showed 100 % infestation with Puccinia.

Example 3.2: Action against Phytophthora on tomato plants

a) Residual-protective action

After 3 weeks' growing period, tomato plants were sprayed with a spray liquor (0.02 % of active ingredient) prepared from a wettable powder of the active ingredient. After 24 hours, the treated plants were infected with a Sporangia suspension of the fungus. Fungal infestation was assessed after the infected plants had been incubated for 5 days at 90-100 % relative atmospheric humidity and 20°C.

b) Systemic action

After 3 weeks' growing period, a spray liquor (0.006 % of active ingredient relative to the soil volume) prepared from a wettable powder of the active ingredient, was poured onto tomato plants. Care was taken that the spray liquor did not come into contact with the above-ground parts of the plants. After 48 hours, the treated plants were infected with a Sporangia suspension of the fungus. Fungal infestation was assessed after the infected plants had been incubated for 5 days at 90-100 % relative atmospheric humidity and 20°C.

In both experiments, no fungal infestation was observed during the evaluation, whereas the infected control plants showed complete infestation.

Example 3.3: Action against Plasmopara viticola on vines

Residual-protective action

Vine seedlings in the 4-5 leaf stage were sprayed with a spray liquor (0.006 % of active ingredient) prepared from a wettable powder of the active ingredient. After 24 hours, the treated plants are infected with a

Sporangia suspension of the fungus. After the plants have been incubated for 6 days at 95-100 % relative atmospheric humidity and 20°C, the fungal infestation is assessed.

In contrast to the untreated, infected control plants where fungal infestation was 100 %, the plants which had been treated with active ingredient I were free from infestation.

Example 3.4: Action against Cercospora arachidicola on peanut plants
Residual-protective action

Peanut plants 10-15 cm in height are sprayed with a spray liquor (0.006 % of active ingredient) prepared from a wettable powder of the active ingredient, and, 48 hours later, infected with a Conidia suspension of the fungus. The infected plants are incubated for 72 hours at about 21°C and a high atmospheric humidity and then placed in a greenhouse until the typical leaf spots occur. The fungicidal action is assessed 12 days after the infection with regard to number and size of the spots which occur.

The plants which had been treated with the active ingredient I were free from infestation. The plants treated with soraphen A had no infestation at the end of the evaluation, even at a spraying concentration of 0.002%. In contrast, untreated, infected control plants showed infestation with Cercospora of 100 %.

Example 3.5: Action against Venturia inaequalis on apple shoots
Residual-protective action

Apple seedlings having fresh shoots of 10-20 cm in length are sprayed with a spray liquor (0.02 % of active ingredient) prepared from a wettable powder of the active ingredient. After 24 hours, the treated plants are infected with a Conidia suspension of the fungus. The plants are then incubated for 5 days at 90-100 % relative atmospheric humidity and placed for 10 more days in a greenhouse at 20-24°C. Scab infestation is assessed 15 days after the infection.

The cuttings treated with the active ingredient were free from infestation, but control plants were completely infested.

Example 3.6: Action against Botrytis cinerea on apple fruits

Residual-protective action

Artificially damaged apples are treated by applying dropwise a spray liquor (0.02 % of active ingredient) prepared from a wettable powder of the active ingredient, to the damaged points. The treated fruits are then inoculated with a spore suspension of the fungus and incubated for one week at high atmospheric humidity and about 20°C. In the evaluation, the damaged points which show signs of rot are counted, and the fungicidal action of the test substance is calculated therefrom.

The active ingredient completely inhibited the growth of the fungi. Untreated but infected control plants had, however, 100% Botrytis infestation.

Example 3.7: Action against Erysiphae graminis on barley

a) Residual-protective action

Barley plants approximately 8 cm in height are sprayed with a spray liquor (0.006 % of active ingredient) prepared from a wettable powder of the active ingredient. After 3-4 hours, the treated plants are dusted with Conidia of the fungus. The infected barley plants are placed in a greenhouse at about 22°C, and fungal infestation is assessed after 10 days.

b) Systemic action

A spray liquor (0.002 % of active ingredient relative to the soil volume) prepared from a wettable powder of the active ingredient, is poured onto barley plants approximately 8 cm in height. Care was taken that the spray liquor did not come into contact with the above-ground parts of the plants. After 48 hours, the treated plants are dusted with Conidia of the fungus. The infected barley plants are placed in a greenhouse at about 22°C, and fungal infestation is assessed after 10 days.

In both experiments, the plants were free from infestation, and the control plants were completely infected.

Example 3.8: Action against Rhizoctonia solani (soil-borne fungus on rice plants)

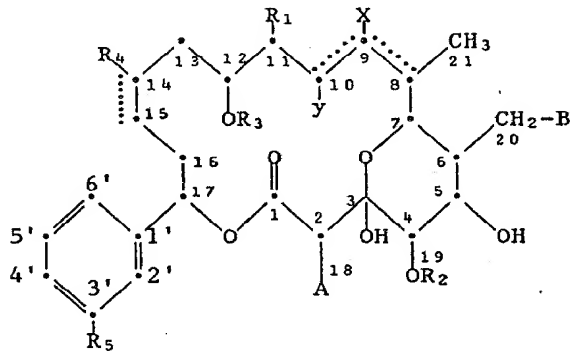
Protective-local soil application

A spray liquor (0.02 % of active ingredient) prepared from a preparation of the active ingredient, is poured onto 12-day old rice plants without contaminating the above-ground parts of the plants. To infect the treated plants, a suspension of mycelium and sclerotia of R. solani is placed on the soil surface. After incubation for 6 days at 27°C (day) and 23°C (night) and 100 % relative atmospheric humidity (humid chamber) in a growth cabinet, the fungal infestation on leaf sheath, leaves and stem is assessed.

No infestation occurred after treatment with the active ingredient, while the infected control plants had complete Rhizoctonia infestation.

WHAT IS CLAIMED IS:

1. Process for producing compounds of the formula I



(I)

in which the macrocyclic ring is either saturated or, alternatively, contains a double bond in the 8,9-position or in the 9,10-position or in the 14,15-position in accordance with the substituent combination below and the substituents $R_1$ to $R_5$, A, B, X and Y are present in the following combined meanings:

| Comp. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | A | B | X | Y | Double bond |
|---|---|---|---|---|---|---|---|---|---|---|
| A | $OCH_3$ | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | H | H | Δ9,10 |
| B | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | H | H | -- |
| C | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | H | H | Δ9,10 |
| D | OH | $CH_3$ | $CH_3$ | H | H | H | H | H | H | -- |
| E | $OCH_3$ | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | OH | H | -- |
| F | $OCH_3$ | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | H | H | -- |
| H | $OCH_3$ | $CH_3$ | H | H | H | $CH_3$ | H | H | OH | Δ8,9 |
| J | $OCH_3$ | H | $CH_3$ | H | H | $CH_3$ | H | H | H | -- |
| M | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | OH | H | -- |
| N | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | H | $OCH_3$ | Δ9,10 |
| Q | $OCH_3$ | $CH_3$ | $CH_3$ | H | H | H | H | H | H | -- |
| R | OH | $CH_3$ | H | H | H | H | H | H | H | -- |
| S | OH | $CH_3$ | H | H | H | $CH_3$ | H | H | H | Δ9,10 |
| T | OH | $CH_3$ | H | H | H | $CH_3$ | H | H | H | -- |
| U | $OCH_3$ | $CH_3$ | $CH_3$ | OH | H | $CH_3$ | H | H | H | Δ9,10 |
| V | $OCH_3$ | $CH_3$ | H | H | H | $CH_3$ | H | H | H | Δ9,10 |
| X | OH | $CH_3$ | H | H | OH | $CH_3$ | H | H | H | Δ9,10 |
| Y | =O | $CH_3$ | H | H | H | $CH_3$ | H | OH | H | -- |
| Z | $OCH_3$ | $CH_3$ | H | OH | H | $CH_3$ | H | H | H | Δ9,10 |
| β | $OCH_3$ | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | H | H | Δ9,10 |
| γ | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | 9,10-epoxy | | -- |
| δ | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | H | H | Δ9,10 |
| ζ | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | OH | H | H | Δ9,10 |
| η | OH | $CH_3$ | $CH_3$ | OH | H | $CH_3$ | H | H | H | Δ9,10 |
| κ | OH | $CH_3$ | H | H | H | $CH_3$ | H | H | H | Δ9,10 |
| μ | $OCH_3$ | $CH_3$ | H | H | H | $CH_3$ | H | H | OH | -- |
| ν | OH | $CH_3$ | $CH_3$ | H | H | $CH_3$ | H | OH | H | Δ14,15 |
| ξ | OH | $CH_3$ | H | H | H | $CH_3$ | H | H | H | Δ9,10 |
| ο | $OCH_3$ | $CH_3$ | $CH_3$ | OH | H | $CH_3$ | H | H | H | Δ9,10 |
| π | OH | $CH_3$ | H | H | H | $CH_3$ | H | H | H | Δ9,10 |
| ρ | OH | $CH_3$ | H | H | H | $CH_3$ | H | OH | H | -- |
| σ | $OCH_3$ | $CH_3$ | $CH_3$ | OH | H | $CH_3$ | H | H | H | Δ9,10 |

including their stereoisomers, wherein, alternatively, a strain

Sorangium cellulosum "So ce 26" (NCIB 12411),

Sorangium cellulosum "So ce 139" (DSM 5397),

Sorangium cellulosum "So ce 170" (DSM 4795),

Sorangium cellulosum "So ce 191" (DSM 4796),

Sorangium cellulosum "So ce 192" (DSM 4797),

Sorangium cellulosum "So ce 231" (DSM 5393) or

Sorangium cellulosum "So ce 242" (DSM 5414)

or a culture derivable from the above, is cultured in a suitable nutrient medium by aerobic culturing and, if desired, the compounds of the formula I obtained are subsequently separated, with the proviso that the strain "So ce 26" (NCIB 12411) is not used to obtain the compounds IA and IB.

2. A process according to claim 1, which comprises culturing one of the seven strains mentioned of Sorangium cellulosum in a culture medium containing at least one assimilatable C source and N source in each case and suitable inorganic salts at 10-40°C in the presence or absence of an adsorbent resin, then extracting the culture broth or the adsorbent resin filtered off with a suitable solvent phase, concentrating the solution obtained and purifying the residue left, as far as desired, by chromatography and/or recrystallization.

3. A process according to claim 2, wherein the fermentation is carried out at 10-35°C with one of the strains
"So ce 170" (DSM 4795)
"So ce 191" (DSM 4796)
"So ce 192" (DSM 4797).

4. The process products obtained by the process according to claim 1.

5. The culture media containing the process products which is obtained by the process according to claim 1 and can be used for agrochemical purposes.

6. Agrochemically usable crude extracts from the culture medium of the process according to claim 1.

7. A macrocyclic compound of formula I according to claim 1 and its stereroisomers in pure form, with the exception of the compounds IA and IB.

8. The compound IY and the compound I ρ (= rho) according to claim 1 in pure form.

9. An agent for combating or preventing plant diseases which contains a process product of the formula I of the process according to claim 1 as active ingredient, with the exception of the compounds IA and IB.

10. The use of the general process products obtained by the process according to claim 1 for combating or preventing plant diseases, with the exception of compounds IA and IB.

11. The use according to claim 10, wherein the process products are those of the formula I or their stereoisomers.

12. A microorganism which is capable of producing a compound of the formula I according to claim 1, with the exception of the microorganism Sorangium(Polyangium) cellulosum "So ce 26" (NCIB 12411) or a mutant or a recombinant of this latter.

13. A biologically pure culture selected from Sorangium cellulosum "So ce 139" (DSM 5397),
Sorangium cellulosum "So ce 170" (DSM 4795)
Sorangium cellulosum "So ce 191" (DSM 4796),
Sorangium cellulosum "So ce 192" (DSM 4797),
Sorangium cellulosum "So ce 231" (DSM 5393)
and Sorangium cellulosum "So ce 242" (DSM 4794),
which is capable of producing at least one compound of the formula I according to claim 1.

14. A biologically derivable culture of one of the microorganisms according to claim 13 which is capable of producing a compound of the formula I.

15. A biologically derivable culture according to claim 1 which is capable of producing at least two compounds of the formula I, including the compound A.

Fetherstonhaugh & Co.
Ottawa, Canada
Patent Agents

FO 7.5/PK/kg*

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FIGURE 1

Separation process for isolating Soraphens I




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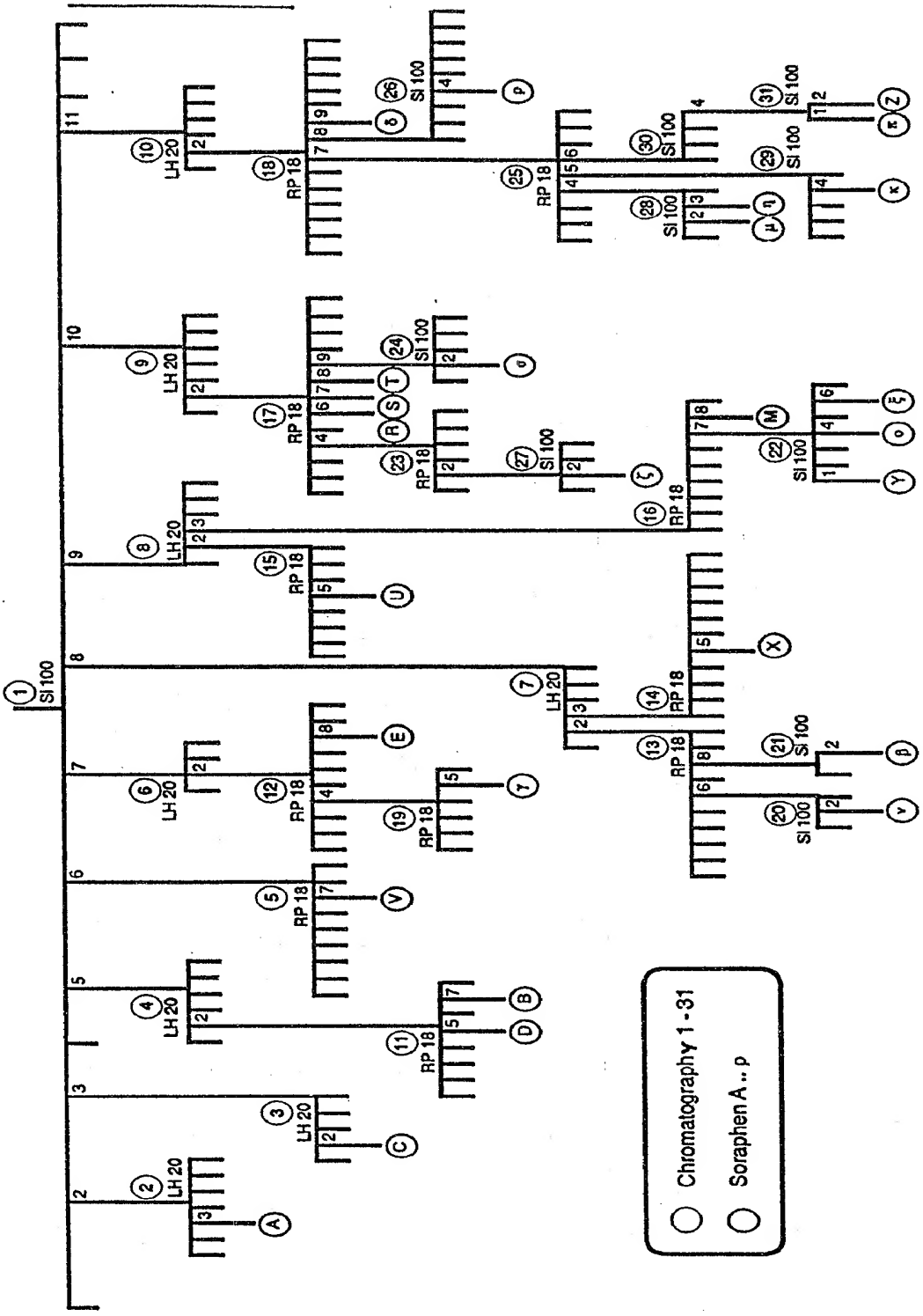
1330203

FIGURE 2

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